

**NUCLEIC ACID MOLECULES AND OTHER MOLECULES ASSOCIATED WITH  
THE METHIONINE SYNTHESIS AND DEGRADATION PATHWAYS**

**CROSS REFERENCE TO RELATED APPLICATIONS**

This application claims the benefit of applications No. 60/067000 filed November 24, 1997, No. 60/066,873 filed November 25, 1997, No. 60/069472 filed December 09, 1997, No. 60/074,201 filed February 10, 1998, No. 60/074282 filed February 10, 1998, No. 60/074280 filed February 10, 1998, No. 60/074281 filed February 10, 1998, No. 60/074566 filed February 12, 1998, No. 60/074567 filed February 12, 1998, No. 60/074565 filed February 12, 1998, No. 60/075462 filed February 19, 1998, No. 60/074789 filed February 19, 1998, No. 60/075459 filed February 19, 1998, No. 60/075461 filed February 19, 1998, No. 60/075464 filed February 19, 1998, No. 60/075460 filed February 19, 1998, No. 60/075463 filed February 19, 1998, No. 60/077231 filed March 09, 1998, No. 60/077229 filed March 09, 1998, No. 60/077230 filed March 09, 1998, No. 60/078031 filed March 16, 1998, No. 60/078368 filed March 18, 1998, No. 60/080844 filed April 07, 1998, No. 60/083067 filed April 27, 1998, "Nucleic Acid Molecules and Other Molecules Associated with Plants" docket No. 38-21(15348)A filed April 29, 1998, No. 60/083387 filed April 29, 1998, No. 60/083388 filed April 29, 1998, No. 60/083389 filed April 29, 1998, "Nucleic Acid Molecules and Other Molecules Associated with the Ethylene Biosynthetic Pathway" docket No. 04983.0018/38-21(15097)A filed May 08, 1998, No. 60/085,245 filed May 13, 1998, No. 60/085224 filed May 13, 1998, No. 60/085223 filed May 13, 1998, No. 60/085222 filed May 13, 1998, No. 60/086186 filed May 21, 1998, No. 60/086,339 filed May 21, 1998, No. 60/086187 filed May 21, 1998, No. 60/086185 filed May 21, 1998, No. 60/086184 filed May 21, 1998, No. 60/086183 filed May 21, 1998, No. 60/086188 filed May 21, 1998, No. 60/089,524 filed June 16, 1998, No. 60/089,810 filed June 18, 1998, No.

60/089,814 filed June 18, 1998, "Nucleic acid molecules and other molecules associated with the Plant Sugar and Nitrogen Transporters Pathway" docket No. 04983.0043/38-21(15412)A filed June 30, 1998, No. 60/092,036 filed July 08, 1998, No. 60/099667 filed September 09, 1998, No. 60/099668 filed September 09, 1998, No. 60/099670 filed September 09, 1998, No. 60/099697 filed September 09, 1998, No. 60/100674 filed September 16, 1998, No. 60/100673 filed September 16, 1998, No. 60/100672 filed September 16, 1998, No. 60/101132 filed September 21, 1998, No. 60/101130 filed September 21, 1998, "Nucleic acid molecules and other molecules associated with Plants" docket No. 38-21(15459)A filed September 21, 1998, No. 60/101344 filed September 22, 1998, No. 60/101347 filed September 22, 1998, No. 60/101343 filed September 22, 1998, No. 60/104,126 filed October 13, 1998, No. 60/104,128 filed October 13, 1998, No. 60/104,127 filed October 13, 1998, No. 60/104,124 filed October 13, 1998, "Nucleic Acid Molecules and Other Molecules Associated with Plants" docket No. 38-21(15445)A filed November 18, 1998 and "Nucleic Acid Molecules and other Molecules associated with Plants" docket No. 38-21(15592) filed November 18, 1998 hereby incorporated by reference herein in their entirety.

### **FIELD OF THE INVENTION**

The present invention is in the field of plant biochemistry. More specifically the invention relates to nucleic acid sequences from plant cells, in particular, DNA sequences from maize and soybean plants associated with the methionine pathway. The invention encompasses nucleic acid molecules that encode proteins and fragments of proteins. In addition, the invention also encompasses proteins and fragments of proteins so encoded and antibodies capable of binding these proteins or fragments. The invention also relates to methods of using the nucleic acid molecules, proteins and fragments of proteins and antibodies, for example for genome

mapping, gene identification and analysis, plant breeding, preparation of constructs for use in plant gene expression and transgenic plants.

## **BACKGROUND OF THE INVENTION**

### **I. METHIONINE SYNTHESIS PATHWAY**

The amino acid, L-methionine, is synthesized in higher plants via a pathway that starts with L-aspartate. This pathway has been studied (Azevedo *et al.*, *Phytochemistry* 46:395-419 (1997), the entirety of which is herein incorporated by reference). L-methionine is one of four so-called aspartate-derived amino acids (along with L-lysine, L-threonine and L-isoleucine)(Mifflin *et al.*, In: *Nitrogen Assimilation in Plants*, Hewitt *et al.*, (eds.), Academic Press, New York, 335 (1997); Bryan, In: *The Biochemistry of Plants*, Mifflin (ed.), Academic Press, New York, 403 (1980); Lea *et al.*, In: *The Chemistry and Biochemistry of Amino Acids*, Barrett *et al.*, (eds.), London, 5:197 (1985); Bryan, In: *The Biochemistry of Plants*, Mifflin *et al.*, (eds.), Academic Press, San Diego, 16:161 (1990), all of which are herein incorporated by reference in their entirety).

The methionine-specific part of the aspartate pathway includes the following enzymes: aspartate kinase (EC 2.7.2.4), aspartate-semialdehyde dehydrogenase (EC 1.2.1.11), homoserine dehydrogenase (EC 1.1.1.3), homoserine kinase (EC 2.7.1.39), cystathionine  $\gamma$ -synthase (EC 4.2.99.9), cystathionine  $\beta$ -lyase (EC 4.4.1.8) and methionine synthase (EC 2.1.1.14).

Aspartate kinase catalyzes the first reaction of the pathway in which aspartate is converted to  $\beta$ -aspartyl phosphate. This enzyme has been isolated and characterized from plant sources including maize, barley, carrot, pea and soybean. These studies have revealed that there are multiple isoenzymes of aspartate kinase and the isoenzymes differ with respect to both

feedback inhibition sensitivity and expression profile (tissue and developmental stage).

Feedback inhibition is mediated by lysine and threonine. Transgenic plants which express an unregulated aspartate kinase have demonstrated increased flux through the aspartate pathway.

Pathway regulation is reported to be exerted, at least in part, via control of this enzyme's activity.

Aspartate semialdehyde dehydrogenase catalyses the second pathway reaction and converts  $\beta$ -aspartyl phosphate to aspartate semialdehyde via an NADPH-dependent reaction. Gengenbach *et al.*, *Crop Science* 18:472-476 (1978), the entirety of which is herein incorporated by reference, report the isolation of aspartate semialdehyde dehydrogenase from maize suspension culture cells. These suspension cultures did not exhibit feedback inhibition of the enzyme in the presence of aspartate-derived amino acids, with the exception of methionine, for which some feedback sensitivity was observed. Aspartate semialdehyde dehydrogenase enzyme activity has been reported in maize shoot, maize root and maize kernel (Gengenbach *et al.*, *Crop Science* 18:472-476 (1978)).

Homoserine dehydrogenase catalyzes the next step of the pathway in which homoserine is generated from aspartate semialdehyde in a reaction requiring NADH or NADPH.

Homoserine dehydrogenase enzyme has been studied in higher plants and multiple isoenzyme forms have been reported (Bryan *et al.*, *Biochemistry and Biophysics Research Communications* 41:1211-1217 (1970); Gengenbach *et al.*, *Crop Science* 18:472-476 (1978); Dotson *et al.*, *Plant Physiology* 91:1602-1608 (1989); Dotson *et al.*, *Plant Physiology* 93:98-104 (1989); Azevedo *et al.*, *Phytochemistry* 31:3725-3730 (1992); Azevedo *et al.*, *Phytochemistry* 31:3731-3734 (1992); Brennecke *et al.*, *Phytochemistry* 41:707 (1996); Aarnes, *Plant Science Letters* 9:137-145 (1977); Bright *et al.*, *Biochemical Genetics* 200:229-243 (1982); Aruda *et al.*, *Plant Physiology*



76:442-446 (1984); Lea *et al.*, In: *Barley: Genetics, Molecular Biology and Biotechnology* Shewrey (ed.), CAB International, Oxford 181 (1992); Davies *et al.*, *Plant Science Letters* 9:323-332 (1977); Davies *et al.*, *Plant Physiology* 62:536-541 (1978); Matthews *et al.*, *Zeitschrift für Naturforschung, Section Bioscience* 34:1177-1185 (1979); Relton *et al.*, *Biochimica et Biophysica Acta* 953:48-60 (1988); Aarnes *et al.*, *Phytochemistry* 13:2717-2724 (1974); Lea *et al.*, *FEBS Letters* 98:165-168 (1979); Matthews *et al.*, *Canadian Journal of Botany* 57:299-304 (1979), all of which references are incorporated herein in their entirety). The isoenzymes have been found to differ with respect to sensitivity to threonine-mediated feedback inhibition, with both sensitive and insensitive forms being isolated from maize suspension cultures and seedlings (Miflin *et al.*, In: *Nitrogen Assimilation of Plants*, Hewitt *et al.*, (eds.), Academic Press, New York, 335 (1997); Bryan, In: *The Biochemistry of Plants*, Miflin (ed.), Academic Press, New York, 5:403 (1980)).

There is evidence that plants also possess a bifunctional enzyme with both aspartate kinase and homoserine dehydrogenase activities (Lea *et al.*, In: *The Chemistry and Biochemistry of Amino Acids*, Barrett *et al.* (eds), London, 5:197 (1985), the entirety of which is herein incorporated by reference; Bryan, In: *The Biochemistry of Plants*, Miflin (ed.), Academic Press, New York, 5:161 (1990), the entirety of which is herein incorporated by reference). Clones of these bifunctional enzymes have been isolated from *Arabidopsis thaliana* (Giovanelli *et al.*, In: *The Biochemistry of Plants*, Miflin (ed.), Academic Press, New York 453 (1990), the entirety of which is herein incorporated by reference) carrot (Giovanelli *et al.*, *Plant Physiology* 90:1584-1599 (1989), the entirety of which is herein incorporated by reference), maize (Singh *et al.*, *Amino Acids* 7:165-168 (1994), the entirety of which is herein incorporated by reference) and soybean (Matthews *et al.*, In: *Biosynthesis and Molecular Regulation of Amino Acids in Plants*, p

294, Singh *et al.* (eds.), American Society of Plant Physiologists, Rockville, MD (1992), the entirety of which is herein incorporated by reference).

The next reported enzymatic step leading to methionine biosynthesis in higher plants is the final common reaction shared by other amino acid end products (threonine and isoleucine). The reaction is catalyzed by homoserine kinase and it generates *O*-phosphohomoserine from homoserine, with ATP serving as the phosphate donor. Exceptions are *Pisum sativum* and *Lathyrus sitivus* which synthesize *O*-acetylhomoserine and *O*-oxalylhomoserine, respectively (Thomas and Surdin-Kerjan, *Microbiol. Mol. Biol. Rev.* 61:503-532 (1997), the entirety of which is herein incorporated by reference). Enteric bacteria use *O*-succinylhomoserine, while several gram-positive bacteria, yeasts and fungi use *O*-acetylhomoserine (formed using homoserine *O*-acetyltransferase (EC 2.3.1.31) (Thomas and Surdin-Kerjan, *Microbiol. Mol. Biol. Rev.* 61:503-532 (1997))). Homoserine kinase has been reported from multiple higher plant sources (Galili, *The Plant Cell* 7:899-906 (1995), the entirety of which is herein incorporated by reference; Rees *et al.*, *Biochemical Journal* 309:999-1107 (1995), the entirety of which is herein incorporated by reference; Bryan *et al.*, *Biochemistry and Biophysics Research Communications* 41:1211-1217 (1970), the entirety of which is herein incorporated by reference; Gengenbach *et al.*, *Crop Science* 18:472-476 (1978), Dotson *et al.*, *Plant Physiology* 91:1602-1608 (1989), the entirety of which is herein incorporated by reference; Dotson *et al.*, *Plant Physiology* 93:98-104 (1989), the entirety of which is herein incorporated by reference). Homoserine kinase isolated from barley and wheat has not been reported to exhibit aspartate-derived amino acid feedback inhibition (Gengenbach *et al.*, *Crop Science* 18:472-476 (1978); Dotson *et al.*, *Plant Physiology* 93:98-104 (1989)). It has been reported that homoserine kinase exhibits feedback regulation in the dicots, pea (Rees *et al.*, *Biochemical Journal* 309:999-1007 (1995), the entirety of which is herein

incorporated by reference) and radish (Bryan *et al.*, *Biochemistry and Biophysics Research Communications* 41:1211-1217 (1970)). Bacterial and yeast homologues have been reported (Azevedo *et al.*, *Phytochemistry* 31:3725-3730 (1992); Azevedo *et al.*, *Phytochemistry* 31:3731-3734 (1992); Brennecke *et al.*, *Phytochemistry* 41:707 (1996); Aarnes, *Plant Science Letters* 9:137-145 (1977)).

Sulfur, in yeast, is incorporated into *O*-acetylhomoserine resulting in homocysteine. This reaction is catalyzed by the *O*-acetylhomoserine sulfhydrylase (EC 4.2.99.10) (also known as *O*-acethomoserine (thiol)-lyase). *O*-acetylhomoserine sulfhydrylase has been reported to be a homotetramer with a molecular weight of 200,000. *O*-acetylhomoserine sulfhydrylase has also been reported to bind four molecules of pyridoxal phosphate (Thomas and Surdin-Kerjan, *Microbiol. Mol. Biol. Rev.* 61:503-532 (1997)).

In higher plants, the sulfur atom from cysteine and the carbon backbone derived from aspartate used to synthesize methionine are reported to be catalyzed by pyridoxal 5'-phosphate (PLP) dependent enzymes (Ravanel *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 95:7805-7812 (1998), the entirety of which is herein incorporated by reference). The amino acid composition of the *O*-acetylhomoserine sulfhydrylase has also been reported to share sequence similarities to the *E. coli* cystathionine  $\gamma$ -synthase and cystathionine  $\beta$ -lyase and cystathionine  $\gamma$ -lyase from *Saccharomyces cerevisiae* and rats. All of these enzymes thus appear to belong to one protein family, whose members have evolved from an ancestral pyridoxal phosphate enzyme (Thomas and Surdin-Kerjan, *Microbiol. Mol. Biol. Rev.* 61:503-532 (1997)).

In yeast, the synthesis of cysteine from homocysteine has been reported to require two successive steps,  $\beta$  addition and  $\gamma$  elimination. Cystathionine  $\beta$ -synthase (EC 4.2.1.22) has been reported to catalyze the first reaction where homocysteine and serine yield cystathionine. In *S.*

*cervisiae*, cystathionine  $\beta$ -synthase is encoded by STR4. STR4 encodes a polypeptide of 506 residues which shows extensive sequence similarity to its functional analog in rats. The rat analog has been reported to contain an additional amino-terminal extension of 60 residues. Moreover, the two enzymes have been reported to be closely related to the cyteine synthase from enteric bacteria and plants (Thomas and Surdin-Kerjan, *Microbiol. Mol. Biol. Rev.* 61:503-532 (1997)).

Cystathionine  $\gamma$ -lyase (EC 4.4.1.1) catalyzes the  $\gamma$  cleavage of cystationine in yeast, the second reported step of the biosynthesis of cysteine from homocysteine. Cystathionine  $\gamma$ -lyase has been reported to have a molecular weight of about 194,000kd. In *S. cerevisiae*, cystathionine  $\gamma$ -lyase is encoded by STR1. A mutation in the *S. cerevisiae* cystathionine  $\gamma$ -lyase gene leads to a nutritional requirement for cysteine or glutathione. The yeast cystathionine  $\gamma$ -lyase belongs to a protein family which includes a functional analog in rats, a Met25p from yeast and cystathionin  $\beta$ -lyase and cystathionin  $\gamma$ -synthase from *E. coli* (Thomas and Surdin-Kerjan, *Microbiol. Mol. Biol. Rev.* 61:503-532 (1997)).

Cystathionine  $\gamma$ -synthase (also known as *O*-succinylhomoserine (thio)-lyase, E.C. 4.2.99.9) catalyzes the first reported reaction which is unique to methionine biosynthesis, thereby committing aspartate pathway flux toward this amino acid. In this reaction, *O*-phosphohomoserine and cysteine serve as substrates for the production of cystathionine. Cystathionine  $\gamma$ -synthase has not been reported to be regulated by aspartate-derived amino acids feedback inhibition (Bright *et al.*, *Biochemical Genetics* 20:229-243 (1982); Arruda *et al.*, *Plant Physiology* 76:442-446 (1984)). Cystathionine  $\gamma$ -synthase has however, been reported to be sensitive to product inhibition by orthophosphate (Lea *et al.*, *Barley: Genetics, Molecular*

*Biology and Biotechnology*, Shewrey (ed.), CAB International, Oxford, 181 (1992); Davies *et al.*, *Plant Science Letters* 9:323-332 (1977)). Cloned cystathionine  $\gamma$ -synthase have been reported from *Arabidopsis thaliana* (Davies *et al.*, *Plant Physiology* 62:536-541 (1978)). It has been reported that methionine levels are modulated via regulation of cystathionine-synthase (Matthews *et al.*, *Zeitschrift für Naturforschung, Section Bioscience* 34:1177-1185 (1979-2724 (1974); Lea *et al.*, *FEBS Letters* 98:165 (1979), all of which references are incorporated herein in their entirety).

Cystathionine  $\beta$ -lyase catalyzes the next reaction in the biosynthesis of methionine. This reaction generates homocysteine, pyruvate and ammonia from the enzymatic decomposition of cystathionine. Evidence for isoenzymes which differ with respect to cellular localization have been reported for barley (Matthews *et al.*, *Canadian Journal of Botany* 57:299-304 (1979)) and spinach (Rognes *et al.*, *Nature* 287:357-359 (1980), the entirety of which is herein incorporated by reference).

De novo synthesis of methionine from homocysteine uses a methyl group which originates from single-carbon metabolism. In this metabolism, derivatives of tetrahydrofolate transfer one-carbon groups at the oxidation levels of methanol, formaldehyde and formate to acceptor molecules. Single-carbon derivatives of tetrahydrofolate are required for the biosynthesis of methionine, purine nucleotides and thymidylate as well as for the synthesis of N-formylmethionine in the mitochondrion. *S. cerevisiae* possesses two complete sets of folate interconversion enzymes, one located in the cytosol (methionyl-tRNA synthetase, EC 6.1.1.10) and the other located in the mitochondrion (methionyl t-RNA synthetase, EC 6.1.1.10) (Thomas and Surdin-Kerjan, *Microbiol. Mol. Biol. Rev.* 61:503-532 (1997)) and in plants including the

chloroplast (Menand *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)*, 95:11014-11019 (1998), the entirety of which is herein incorporated by reference).

Methionine synthase generates methionine from homocysteine by a methylation reaction and thus represents the final step of the methionine biosynthetic pathway. Methionine synthase is also sometimes referred to as 5-methyltetrahydropteroyltriglutamate-homocysteine-S-methyltransferase. N-methyltetrahydrofolate serves as the methyl donor in this reaction, which occurs in the absence of cobalamin (Giovannelli *et al.*, *Plant Physiology* 90:1577-1583 (1989), the entirety of which is herein incorporated by reference; Green *et al.*, *Crop Science* 14:827-830 (1974), the entirety of which is herein incorporated by reference).

## II. METHIONINE DEGRADATION PATHWAY

Plants contain a pathway for the degradation of L-methionine. This degradation pathway includes the following enzymes: methionine adenosyltransferase (EC 2.5.1.6), methionine S-methyltransferase (EC 2.1.1.12), adenosylmethionine hydrolase (EC 3.3.1.2), homocysteine S-methyltransferase (EC 2.1.1.10) and S-adenosyl-methionine decarboxylase (EC 4.1.1.50).

The reported first step in the catabolism of methionine is the ATP-dependent conversion to S-adenosylmethionine (AdoMet), which is catalyzed by the enzyme methionine adenosyltransferase, also known as S-adenosylmethionine synthetase. Methionine adenosyltransferase enzyme has been characterized from several plant sources (Aarnes, *Plant Science Letters* 10:381 (1977), the entirety of which is herein incorporated by reference; Mathur *et al.*, *Biochimica and Biophysica Acta* 1078:161-170 (1991), the entirety of which is herein incorporated by reference; Kim *et al.*, *Journal of Biochemical and Molecular Biology* 28:100 (1995), the entirety of which is herein incorporated by reference) and nucleic acid molecules (genomic and cDNA) have also been obtained from a variety of sources (Izhaki *et al.*, *Plant*

*Physiology* 108:841-842 (1995), the entirety of which is herein incorporated by reference; Espartero *et al.*, *Molecular Biology Plant* 25:217-237 (1994), the entirety of which is herein incorporated by reference). Regulation of methionine adenosyltransferase activity has been observed for the enzyme from *Glycine max* (soybean). In *Glycine max*, methionine adenosyltransferase was reportedly inhibited by S-adenosylmethionine (Kim *et al.*, *Journal of Biochemical and Molecular Biology* 28:100 (1995). Studies have also reported that the levels of methionine adenosyltransferase appear to fluctuate in response to hormonal or environmental conditions such as gibberellic acid (Mathur *et al.*, *Biochimica and Biophysica Acta* 1162:289-290 (1993), the entirety of which is herein incorporated by reference; Mathur *et al.*, *Biochimica and Biophysica Acta* 1137:338-348 (1992), the entirety of which is herein incorporated by reference), salt stress (Espartero *et al.*, *Molecular Biology Plant* 25:217-227 (1994) the entirety of which is herein incorporated by reference) and wounding (Kim *et al.*, *Plant Cell Reports* 13:340 (1994), the entirety of which is herein incorporated by reference). It has also been reported that methionine adenosyltransferase may play a role in the lignification process (Peleman *et al.*, *Plant Cell* 1:81 (1989), the entirety of which is herein incorporated by reference).

AdoMet is further catabolized by several enzymes and has been reported to serve a variety of metabolic functions including that of a methyl donor (Cossins, *The Biochemistry of Plants* 11:317 Devis (ed.), Academic Press, San Diego (1987), the entirety of which is herein incorporated by reference) that of a precursor for polyamine biosynthesis (Tiburico *et al.*, *The Biochemistry of Plants* 16:283 (1990), the entirety of which is herein incorporated by reference) and that of a precursor for ethylene biosynthesis (Kende, *Plant Physiology* 91:1-4 (1989), the entirety of which is herein incorporated by reference; Flurh *et al.*, *Critical Review of Plant*

*Science* 15:479 (1996), the entirety of which is herein incorporated by reference). In each case, enzymes are present to regenerate methionine from the sulfur-containing backbone resulting in no net loss of methionine.

An enzyme involved in AdoMet catabolism is adenosylmethionine hydrolase (EC 3.3.1.2) which converts AdoMet to methylthioadenosine and L-homoserine. L-homoserine is further metabolized during the biosynthesis of polyamines and ethylene and methylthioadenosine is recycled to methionine. In yeast, a form of adenosylmethionine hydrolase (EC 3.1.1.1) has been reported (<http://www.ncbi.nlm.nih.gov/htbin-post/Entrez/query> (1998)).

Another enzyme for which AdoMet is a substrate for is homocysteine S-methyltransferase. Homocysteine S-methyltransferase catalyzes the combination of AdoMet, with L-homocysteine to produce both S-adenosyl-L-homocysteine and L-methionine. Another enzyme has been described which generates S-adenosyl-L-homocysteine from AdoMet. This enzyme is called methionine S-methyltransferase and it catalyzes the reaction in which S-adenosyl-L-homocysteine reacts with L-methionine to generate S-adenosyl-L-homocysteine and S-methyl-L-methionine. AdoMet can also be decarboxylated by adenosyl methionine decarboxylase, which generates (5-deoxy-5-adenosyl) (3-aminopropyl) methylsulfonium salt.

### **III. EXPRESSED SEQUENCE TAG NUCLEIC ACID MOLECULES**

Expressed sequence tags, or ESTs are randomly sequenced members of a cDNA library (or complementary DNA)(McCombie *et al.*, *Nature Genetics* 1:124-130 (1992); Kurata *et al.*, *Nature Genetics* 8:365-372 (1994); Okubo *et al.*, *Nature Genetics* 2:173-179 (1992), all of which references are incorporated herein in their entirety). The randomly selected clones comprise insets that can represent a copy of up to the full length of a mRNA transcript.



Using conventional methodologies, cDNA libraries can be constructed from the mRNA (messenger RNA) of a given tissue or organism using poly dT primers and reverse transcriptase (Efstratiadis *et al.*, *Cell* 7:279-3680 (1976), the entirety of which is herein incorporated by reference; Higuchi *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 73:3146-3150 (1976), the entirety of which is herein incorporated by reference; Maniatis *et al.*, *Cell* 8:163-182 (1976) the entirety of which is herein incorporated by reference; Land *et al.*, *Nucleic Acids Res.* 9:2251-2266 (1981), the entirety of which is herein incorporated by reference; Okayama *et al.*, *Mol. Cell. Biol.* 2:161-170 (1982), the entirety of which is herein incorporated by reference; Gubler *et al.*, *Gene* 25:263-269 (1983), the entirety of which is herein incorporated by reference).

Several methods may be employed to obtain full-length cDNA constructs. For example, terminal transferase can be used to add homopolymeric tails of dC residues to the free 3' hydroxyl groups (Land *et al.*, *Nucleic Acids Res.* 9:2251-2266 (1981), the entirety of which is herein incorporated by reference). This tail can then be hybridized by a poly dG oligo which can act as a primer for the synthesis of full length second strand cDNA. Okayama and Berg, *Mol. Cell. Biol.* 2:161-170 (1982), the entirety of which is herein incorporated by reference, report a method for obtaining full length cDNA constructs. This method has been simplified by using synthetic primer-adapters that have both homopolymeric tails for priming the synthesis of the first and second strands and restriction sites for cloning into plasmids (Coleclough *et al.*, *Gene* 34:305-314 (1985), the entirety of which is herein incorporated by reference) and bacteriophage vectors (Krawinkel *et al.*, *Nucleic Acids Res.* 14:1913 (1986), the entirety of which is herein incorporated by reference; Han *et al.*, *Nucleic Acids Res.* 15:6304 (1987), the entirety of which is herein incorporated by reference).

These strategies have been coupled with additional strategies for isolating rare mRNA populations. For example, a typical mammalian cell contains between 10,000 and 30,000 different mRNA sequences (Davidson, *Gene Activity in Early Development*, 2nd ed., Academic Press, New York (1976), the entirety of which is herein incorporated by reference). The number of clones required to achieve a given probability that a low-abundance mRNA will be present in a cDNA library is  $N = (\ln(1-P))/(\ln(1-1/n))$  where N is the number of clones required, P is the probability desired and 1/n is the fractional proportion of the total mRNA that is represented by a single rare mRNA (Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press (1989), the entirety of which is herein incorporated by reference).

A method to enrich preparations of mRNA for sequences of interest is to fractionate by size. One such method is to fractionate by electrophoresis through an agarose gel (Pennica *et al.*, *Nature* 301:214-221 (1983), the entirety of which is herein incorporated by reference). Another such method employs sucrose gradient centrifugation in the presence of an agent, such as methylmercuric hydroxide, that denatures secondary structure in RNA (Schweinfest *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 79:4997-5000 (1982), the entirety of which is herein incorporated by reference).

A frequently adopted method is to construct equalized or normalized cDNA libraries (Ko, *Nucleic Acids Res.* 18:5705-5711 (1990), the entirety of which is herein incorporated by reference; Patanjali *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 88:1943-1947 (1991), the entirety of which is herein incorporated by reference). Typically, the cDNA population is normalized by subtractive hybridization (Schmid *et al.*, *J. Neurochem.* 48:307-312 (1987), the entirety of which is herein incorporated by reference; Fagnoli *et al.*, *Anal. Biochem.* 187:364-373 (1990), the

entirety of which is herein incorporated by reference; Travis *et al.*, *Proc. Natl. Acad. Sci (U.S.A.)* 85:1696-1700 (1988), the entirety of which is herein incorporated by reference; Kato, *Eur. J. Neurosci.* 2:704-711 (1990); and Schweinfest *et al.*, *Genet. Anal. Tech. Appl.* 7:64-70 (1990), the entirety of which is herein incorporated by reference). Subtraction represents another method for reducing the population of certain sequences in the cDNA library (Swaroop *et al.*, *Nucleic Acids Res.* 19:1954 (1991), the entirety of which is herein incorporated by reference).

ESTs can be sequenced by a number of methods. Two basic methods may be used for DNA sequencing, the chain termination method of Sanger *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 74:5463-5467 (1977), the entirety of which is herein incorporated by reference and the chemical degradation method of Maxam and Gilbert, *Proc. Nat. Acad. Sci. (U.S.A.)* 74:560-564 (1977), the entirety of which is herein incorporated by reference. Automation and advances in technology such as the replacement of radioisotopes with fluorescence-based sequencing have reduced the effort required to sequence DNA (Craxton, *Methods* 2:20-26 (1991), the entirety of which is herein incorporated by reference; Ju *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 92:4347-4351 (1995), the entirety of which is herein incorporated by reference; Tabor and Richardson, *Proc. Natl. Acad. Sci. (U.S.A.)* 92:6339-6343 (1995), the entirety of which is herein incorporated by reference). Automated sequencers are available from, for example, Pharmacia Biotech, Inc., Piscataway, New Jersey (Pharmacia ALF), LI-COR, Inc., Lincoln, Nebraska (LI-COR 4,000) and Millipore, Bedford, Massachusetts (Millipore BaseStation).

In addition, advances in capillary gel electrophoresis have also reduced the effort required to sequence DNA and such advances provide a rapid high resolution approach for sequencing DNA samples (Swerdlow and Gesteland, *Nucleic Acids Res.* 18:1415-1419 (1990); Smith, *Nature* 349:812-813 (1991); Luckey *et al.*, *Methods Enzymol.* 218:154-172 (1993); Lu *et al.*, *J.*

*Chromatog. A.* 680:497-501 (1994); Carson *et al.*, *Anal. Chem.* 65:3219-3226 (1993); Huang *et al.*, *Anal. Chem.* 64:2149-2154 (1992); Kheterpal *et al.*, *Electrophoresis* 17:1852-1859 (1996); Quesada and Zhang, *Electrophoresis* 17:1841-1851 (1996); Baba, *Yakugaku Zasshi* 117:265-281 (1997), all of which are herein incorporated by reference in their entirety).

ESTs longer than 150 nucleotides have been found to be useful for similarity searches and mapping (Adams *et al.*, *Science* 252:1651-1656 (1991), herein incorporated by reference). ESTs, which can represent copies of up to the full length transcript, may be partially or completely sequenced. Between 150-450 nucleotides of sequence information is usually generated as this is the length of sequence information that is routinely and reliably produced using single run sequence data. Typically, only single run sequence data is obtained from the cDNA library (Adams *et al.*, *Science* 252:1651-1656 (1991). Automated single run sequencing typically results in an approximately 2-3% error or base ambiguity rate (Boguski *et al.*, *Nature Genetics* 4:332-333 (1993), the entirety of which is herein incorporated by reference).

EST databases have been constructed or partially constructed from, for example, *C. elegans* (McCombie *et al.*, *Nature Genetics* 1:124-131 (1992)), human liver cell line HepG2 (Okubo *et al.*, *Nature Genetics* 2:173-179 (1992)), human brain RNA (Adams *et al.*, *Science* 252:1651-1656 (1991); Adams *et al.*, *Nature* 355:632-635 (1992)), *Arabidopsis*, (Newman *et al.*, *Plant Physiol.* 106:1241-1255 (1994)); and rice (Kurata *et al.*, *Nature Genetics* 8:365-372 (1994)).

#### IV. SEQUENCE COMPARISONS

A characteristic feature of a DNA sequence is that it can be compared with other DNA sequences. Sequence comparisons can be undertaken by determining the similarity of the test or query sequence with sequences in publicly available or proprietary databases ("similarity

analysis”) or by searching for certain motifs (“intrinsic sequence analysis”)(e.g. *cis* elements)(Coulson, *Trends in Biotechnology* 12:76-80 (1994), the entirety of which is herein incorporated by reference); Birren *et al.*, *Genome Analysis I*: Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York 543-559 (1997), the entirety of which is herein incorporated by reference).

Similarity analysis includes database search and alignment. Examples of public databases include the DNA Database of Japan (DDBJ)(<http://www.ddbj.nig.ac.jp/>); Genbank (<http://www.ncbi.nlm.nih.gov/Web/Search/Index.html>); and the European Molecular Biology Laboratory Nucleic Acid Sequence Database (EMBL) ([http://www.ebi.ac.uk/ebi\\_docs/embl\\_db/embl-db.html](http://www.ebi.ac.uk/ebi_docs/embl_db/embl-db.html)). Other appropriate databases include dbEST (<http://www.ncbi.nlm.nih.gov/dbEST/index.html>), SwissProt ([http://www.ebi.ac.uk/ebi\\_docs/swisprot\\_db/swisshome.html](http://www.ebi.ac.uk/ebi_docs/swisprot_db/swisshome.html)), PIR (<http://www-nbrt.georgetown.edu/pir/>) and The Institute for Genome Research (<http://www.tigr.org/tdb/tdb.html>)

A number of different search algorithms have been developed, one example of which are the suite of programs referred to as BLAST programs. There are five implementations of BLAST, three designed for nucleotide sequences queries (BLASTN, BLASTX and TBLASTX) and two designed for protein sequence queries (BLASTP and TBLASTN) (Coulson, *Trends in Biotechnology* 12:76-80 (1994); Birren *et al.*, *Genome Analysis I*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York 543-559 (1997)).

BLASTN takes a nucleotide sequence (the query sequence) and its reverse complement and searches them against a nucleotide sequence database. BLASTN was designed for speed, not maximum sensitivity and may not find distantly related coding sequences. BLASTX takes a

nucleotide sequence, translates it in three forward reading frames and three reverse complement reading frames and then compares the six translations against a protein sequence database.

BLASTX is useful for sensitive analysis of preliminary (single-pass) sequence data and is tolerant of sequencing errors (Gish and States, *Nature Genetics* 3:266-272 (1993), the entirety of which is herein incorporated by reference). BLASTN and BLASTX may be used in concert for analyzing EST data (Coulson, *Trends in Biotechnology* 12:76-80 (1994); Birren *et al.*, *Genome Analysis* 1:543-559 (1997)).

Given a coding nucleotide sequence and the protein it encodes, it is often preferable to use the protein as the query sequence to search a database because of the greatly increased sensitivity to detect more subtle relationships. This is due to the larger alphabet of proteins (20 amino acids) compared with the alphabet of nucleic acid sequences (4 bases), where it is far easier to obtain a match by chance. In addition, with nucleotide alignments, only a match (positive score) or a mismatch (negative score) is obtained, but with proteins, the presence of conservative amino acid substitutions can be taken into account. Here, a mismatch may yield a positive score if the non-identical residue has physical/chemical properties similar to the one it replaced. Various scoring matrices are used to supply the substitution scores of all possible amino acid pairs. A general purpose scoring system is the BLOSUM62 matrix (Henikoff and Henikoff, *Proteins* 17:49-61 (1993), the entirety of which is herein incorporated by reference), which is currently the default choice for BLAST programs. BLOSUM62 is tailored for alignments of moderately diverged sequences and thus may not yield the best results under all conditions. Altschul, *J. Mol. Biol.* 36:290-300 (1993), the entirety of which is herein incorporated by reference, describes a combination of three matrices to cover all contingencies. This may improve sensitivity, but at the expense of slower searches. In practice, a single

BLOSUM62 matrix is often used but others (PAM40 and PAM250) may be attempted when additional analysis is necessary. Low PAM matrices are directed at detecting very strong but localized sequence similarities, whereas high PAM matrices are directed at detecting long but weak alignments between very distantly related sequences.

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Homologues in other organisms are available that can be used for comparative sequence analysis. Multiple alignments are performed to study similarities and differences in a group of related sequences. CLUSTAL W is a multiple sequence alignment package that performs progressive multiple sequence alignments based on the method of Feng and Doolittle, *J. Mol. Evol.* 25:351-360 (1987), the entirety of which is herein incorporated by reference. Each pair of sequences is aligned and the distance between each pair is calculated; from this distance matrix, a guide tree is calculated and all of the sequences are progressively aligned based on this tree. A feature of the program is its sensitivity to the effect of gaps on the alignment; gap penalties are varied to encourage the insertion of gaps in probable loop regions instead of in the middle of structured regions. Users can specify gap penalties, choose between a number of scoring matrices, or supply their own scoring matrix for both pairwise alignments and multiple alignments. CLUSTAL W for UNIX and VMS systems is available at: <ftp.ebi.ac.uk>. Another program is MACAW (Schuler *et al.*, *Proteins Struct. Func. Genet.* 9:180-190 (1991), the entirety of which is herein incorporated by reference, for which both Macintosh and Microsoft Windows versions are available. MACAW uses a graphical interface, provides a choice of several alignment algorithms and is available by anonymous ftp at: <ncbi.nlm.nih.gov> (directory/pub/macaw).

Sequence motifs are derived from multiple alignments and can be used to examine individual sequences or an entire database for subtle patterns. With motifs, it is sometimes

possible to detect distant relationships that may not be demonstrable based on comparisons of primary sequences alone. Currently, the largest collection of sequence motifs in the world is PROSITE (Bairoch and Bucher, *Nucleic Acid Research* 22:3583-3589 (1994), the entirety of which is herein incorporated by reference). PROSITE may be accessed via either the ExPASy server on the World Wide Web or anonymous ftp site. Many commercial sequence analysis packages also provide search programs that use PROSITE data.

A resource for searching protein motifs is the BLOCKS E-mail server developed by Henikoff, *Trends Biochem Sci.* 18:267-268 (1993), the entirety of which is herein incorporated by reference; Henikoff and Henikoff, *Nucleic Acid Research* 19:6565-6572 (1991), the entirety of which is herein incorporated by reference; Henikoff and Henikoff, *Proteins* 17:49-61 (1993). BLOCKS searches a protein or nucleotide sequence against a database of protein motifs or "blocks." Blocks are defined as short, ungapped multiple alignments that represent highly conserved protein patterns. The blocks themselves are derived from entries in PROSITE as well as other sources. Either a protein query or a nucleotide query can be submitted to the BLOCKS server; if a nucleotide sequence is submitted, the sequence is translated in all six reading frames and motifs are sought for these conceptual translations. Once the search is completed, the server will return a ranked list of significant matches, along with an alignment of the query sequence to the matched BLOCKS entries.

Conserved protein domains can be represented by two-dimensional matrices, which measure either the frequency or probability of the occurrences of each amino acid residue and deletions or insertions in each position of the domain. This type of model, when used to search against protein databases, is sensitive and usually yields more accurate results than simple motif searches. Two popular implementations of this approach are profile searches such as GCG



program ProfileSearch and Hidden Markov Models (HMMs)(Krough *et al.*, *J. Mol. Biol.* 235:1501-1531, (1994); Eddy, *Current Opinion in Structural Biology* 6:361-365, (1996), both of which are herein incorporated by reference in their entirety). In both cases, a large number of common protein domains have been converted into profiles, as present in the PROSITE library, or HMM models, as in the Pfam protein domain library (Sonnhammer *et al.*, *Proteins* 28:405-420 (1997), the entirety of which is herein incorporated by reference). Pfam contains more than 500 HMM models for enzymes, transcription factors, signal transduction molecules and structural proteins. Protein databases can be queried with these profiles or HMM models, which will identify proteins containing the domain of interest. For example, HMMSW or HMMFS, two programs in a public domain package called HMMER (Sonnhammer *et al.*, *Proteins* 28:405-420 (1997)) can be used.

PROSITE and BLOCKS represent collected families of protein motifs. Thus, searching these databases entails submitting a single sequence to determine whether or not that sequence is similar to the members of an established family. Programs working in the opposite direction compare a collection of sequences with individual entries in the protein databases. An example of such a program is the Motif Search Tool, or MoST (Tatusov *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 91:12091-12095 (1994), the entirety of which is herein incorporated by reference). On the basis of an aligned set of input sequences, a weight matrix is calculated by using one of four methods (selected by the user). A weight matrix is simply a representation, position by position of how likely a particular amino acid will appear. The calculated weight matrix is then used to search the databases. To increase sensitivity, newly found sequences are added to the original data set, the weight matrix is recalculated and the search is performed again. This procedure continues until no new sequences are found.

## SUMMARY OF THE INVENTION

The present invention provides a substantially purified nucleic acid molecule that encodes a maize or soybean enzyme or fragment thereof, wherein said maize or soybean enzyme is selected from the group consisting of: (a) methionine adenosyltransferase, (b) S-adenosyl-methionine decarboxylase, (c) aspartate kinase, (d) aspartate-semialdehyde dehydrogenase, (e) cystathionine gamma-synthase, (f) cystathionine beta-lyase, and (g) 5-methyltetrahydropteroyltriglutamate-homocysteine-S-methyltransferase.

The present invention also provides a substantially purified nucleic acid molecule that encodes a plant methionine pathway enzyme or fragment thereof, wherein the nucleic acid molecule is selected from the group consisting of a nucleic acid molecule that encodes a maize or a soybean methionine adenosyltransferase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean S-adenosylmethionine decarboxylase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean aspartate kinase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean aspartate-semialdehyde dehydrogenase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean *O*-succinylhomoserine (thiol)-lyase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean cystathionine  $\beta$ -lyase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean 5-methyltetrahydropteroyltriglutamate-homocysteine-S-methyltransferase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean adenosylhomocysteinase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean cystathionine  $\beta$ -synthase enzyme or fragment thereof, a nucleic acid molecule that encodes a

maize or a soybean cystathionine  $\gamma$ -lyase enzyme or fragment thereof and a nucleic acid molecule that encodes a maize or a soybean *O*-acetylhomoserine (thiol)-lyase enzyme or fragment thereof.

A substantially purified maize or soybean enzyme or fragment thereof, wherein said maize or soybean enzyme is selected from the group consisting of (a) methionine adenosyltransferase or fragment thereof; (b) S-adenosyl-methionine decarboxylase or fragment thereof; (c) aspartate kinase or fragment thereof; (d) aspartate-semialdehyde dehydrogenase or fragment thereof; (e) cystathionine gamma-synthase or fragment thereof; (f) cystathionine beta-lyase or fragment thereof; and (g) 5-methyltetrahydropteroyltriglutamate-homocysteine-S-methyltransferase or fragment thereof.

The present invention also provides a substantially purified maize or soybean methionine pathway protein or fragment thereof encoded by a first nucleic acid molecule which specifically hybridizes to a second nucleic acid molecule, the second nucleic acid molecule having a nucleic acid sequence selected from the group consisting of a complement of SEQ ID NO: 1 through SEQ ID NO: 3204.

The present invention also provides a substantially purified maize or soybean methionine adenosyltransferase enzyme or fragment thereof encoded by a first nucleic acid molecule which specifically hybridizes to a second nucleic acid molecule, the second nucleic acid molecule having a nucleic acid sequence selected from the group consisting of a complement of SEQ ID NO: 1 through SEQ ID NO: 429 and SEQ ID NO: 1635 through SEQ ID NO: 2479.

The present invention also provides a substantially purified maize or soybean methionine adenosyltransferase enzyme or fragment thereof encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 429 and SEQ ID NO: 1635 through SEQ ID NO: 2479.

The present invention also provides a substantially purified maize or soybean S-adenosylmethionine decarboxylase enzyme or fragment thereof encoded by a first nucleic acid molecule which specifically hybridizes to a second nucleic acid molecule, the second nucleic acid molecule having a nucleic acid sequence selected from the group consisting of a complement of SEQ ID NO: 430 through SEQ ID NO: 857 and SEQ ID NO: 2480 through SEQ ID NO: 2623.

The present invention also provides a substantially purified maize or soybean S-adenosylmethionine decarboxylase enzyme or fragment thereof encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 430 through SEQ ID NO: 857 and SEQ ID NO: 2480 through SEQ ID NO: 2623.

The present invention also provides a substantially purified maize or soybean aspartate kinase enzyme or fragment thereof encoded by a first nucleic acid molecule which specifically hybridizes to a second nucleic acid molecule, the second nucleic acid molecule having a nucleic acid sequence selected from the group consisting of a complement of SEQ ID NO: 858 through SEQ ID NO: 900 and SEQ ID NO: 2624 through SEQ ID NO: 2648.

The present invention also provides a substantially purified maize or soybean aspartate kinase enzyme or fragment thereof encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 858 through SEQ ID NO: 900 and SEQ ID NO: 2624 through SEQ ID NO: 2648.

The present invention also provides a substantially purified maize or soybean aspartate-semialdehyde dehydrogenase enzyme or fragment thereof encoded by a first nucleic acid molecule which specifically hybridizes to a second nucleic acid molecule, the second nucleic acid molecule having a nucleic acid sequence selected from the group consisting of a

complement of SEQ ID NO: 901 through SEQ ID NO: 904 and SEQ ID NO: 2649 through SEQ ID NO: 2654.

The present invention also provides a substantially purified maize or soybean aspartate-semialdehyde dehydrogenase enzyme or fragment thereof encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 901 through SEQ ID NO: 904 and SEQ ID NO: 2649 through SEQ ID NO: 2654.

The present invention also provides a substantially purified maize or soybean *O*-succinylhomoserine (thiol)-lyase enzyme or fragment thereof encoded by a first nucleic acid molecule which specifically hybridizes to a second nucleic acid molecule, the second nucleic acid molecule having a nucleic acid sequence selected from the group consisting of a complement of SEQ ID NO: 905 through SEQ ID NO: 953 and SEQ ID NO: 2655 through SEQ ID NO: 2660.

The present invention also provides a substantially purified maize or soybean *O*-succinylhomoserine (thiol)-lyase enzyme or fragment thereof encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 905 through SEQ ID NO: 953 and SEQ ID NO: 2655 through SEQ ID NO: 2660.

The present invention also provides a substantially purified maize or soybean cystathionine  $\beta$ -lyase enzyme or fragment thereof encoded by a first nucleic acid molecule which specifically hybridizes to a second nucleic acid molecule, the second nucleic acid molecule having a nucleic acid sequence selected from the group consisting of a complement of SEQ ID NO: 954 through SEQ ID NO: 963 and SEQ ID NO: 2661 through SEQ ID NO: 2665.

The present invention also provides a substantially purified maize or soybean cystathionine  $\beta$ -lyase enzyme or fragment thereof encoded by a nucleic acid sequence selected

from the group consisting of SEQ ID NO: 954 through SEQ ID NO: 963 and SEQ ID NO: 2661 through SEQ ID NO: 2665.

The present invention also provides a substantially purified maize or soybean 5-methyltetrahydropteroyltriglutamate-homocysteine-S-methyltransferase enzyme or fragment thereof encoded by a first nucleic acid molecule which specifically hybridizes to a second nucleic acid molecule, the second nucleic acid molecule having a nucleic acid sequence selected from the group consisting of a complement of SEQ ID NO: 964 through SEQ ID NO: 1353 and SEQ ID NO: 2666 through SEQ ID NO: 2992.

The present invention also provides a substantially purified maize or soybean 5-methyltetrahydropteroyltriglutamate-homocysteine-S-methyltransferase enzyme or fragment thereof encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 964 through SEQ ID NO: 1353 and SEQ ID NO: 2666 through SEQ ID NO: 2992.

The present invention also provides a substantially purified maize or adenosylhomocysteinase enzyme or fragment thereof encoded by a first nucleic acid molecule which specifically hybridizes to a second nucleic acid molecule, the second nucleic acid molecule having a nucleic acid sequence selected from the group consisting of a complement of SEQ ID NO: 1354 through SEQ ID NO: 1630 and SEQ ID NO: 2993 through SEQ ID NO: 3199.

The present invention also provides a substantially purified maize or adenosylhomocysteinase enzyme or fragment thereof encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1354 through SEQ ID NO: 1630 and SEQ ID NO: 2993 through SEQ ID NO: 3199.

The present invention also provides a substantially purified maize or cystathionine  $\beta$ -synthase enzyme or fragment thereof encoded by a first nucleic acid molecule which specifically hybridizes to a second nucleic acid molecule, the second nucleic acid molecule having a nucleic acid sequence selected from the group consisting of a complement of SEQ ID NO: 1631 through SEQ ID NO: 1632.

The present invention also provides a substantially purified maize or cystathionine  $\beta$ -synthase enzyme or fragment thereof encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1631 through SEQ ID NO: 1632.

The present invention also provides a substantially purified maize or cystathionine  $\gamma$ -lyase enzyme or fragment thereof encoded by a first nucleic acid molecule which specifically hybridizes to a second nucleic acid molecule, the second nucleic acid molecule having a nucleic acid sequence selected from the group consisting of a complement of SEQ ID NO: 1633 through SEQ ID NO: 1634 and SEQ ID NO: 3203 through SEQ ID NO: 3204.

The present invention also provides a substantially purified maize or cystathionine  $\gamma$ -lyase enzyme or fragment thereof encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1633 through SEQ ID NO: 1634 and SEQ ID NO: 3203 through SEQ ID NO: 3204.

The present invention also provides a substantially purified maize or *O*-acetylhomoserine (thiol)-lyase enzyme or fragment thereof encoded by a first nucleic acid molecule which specifically hybridizes to a second nucleic acid molecule, the second nucleic acid molecule having a nucleic acid sequence selected from the group consisting of a complement of SEQ ID NO: 3200 through SEQ ID NO: 3202.

The present invention also provides a substantially purified maize or *O*-acetylhomoserine (thiol)-lyase enzyme or fragment thereof encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 3200 through SEQ ID NO: 3202.

The present invention also provides a purified antibody or fragment thereof which is capable of specifically binding to a specific maize or soybean enzyme or fragment thereof, wherein said maize or soybean enzyme or fragment thereof is encoded by a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 3204.

The present invention also provides a substantially purified antibody or fragment thereof, the antibody or fragment thereof capable of specifically binding to a maize or a soybean methionine adenosyltransferase enzyme or fragment thereof encoded by a first nucleic acid molecule which specifically hybridizes to a second nucleic acid molecule, the second nucleic acid molecule having a nucleic acid sequence selected from the group consisting of a complement of SEQ ID NO: 1 through SEQ ID NO: 429 and SEQ ID NO: 1635 through SEQ ID NO: 2479 or a substantially purified maize or soybean methionine adenosyltransferase enzyme or fragment thereof encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 429 and SEQ ID NO: 1635 through SEQ ID NO: 2479.

The present invention also provides a substantially purified antibody or fragment thereof, the antibody or fragment thereof capable of specifically binding to a maize or a soybean S-adenosylmethionine decarboxylase enzyme or fragment thereof encoded by a first nucleic acid molecule which specifically hybridizes to a second nucleic acid molecule, the second nucleic acid molecule having a nucleic acid sequence selected from the group consisting of a complement of SEQ ID NO: 430 through SEQ ID NO: 857 and SEQ ID NO: 2480 through SEQ



ID NO: 2623 or a substantially purified maize or soybean S-adenosylmethionine decarboxylase enzyme or fragment thereof encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 430 through SEQ ID NO: 857 and SEQ ID NO: 2480 through SEQ ID NO: 2623.

The present invention also provides a substantially purified antibody or fragment thereof, the antibody or fragment thereof capable of specifically binding to a maize or a soybean aspartate kinase enzyme or fragment thereof encoded by a first nucleic acid molecule which specifically hybridizes to a second nucleic acid molecule, the second nucleic acid molecule having a nucleic acid sequence selected from the group consisting of a complement of SEQ ID NO: 858 through SEQ ID NO: 900 and SEQ ID NO: 2624 through SEQ ID NO: 2648 or a substantially purified maize or soybean aspartate kinase enzyme or fragment thereof encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 858 through SEQ ID NO: 900 and SEQ ID NO: 2624 through SEQ ID NO: 2648.

The present invention also provides a substantially purified antibody or fragment thereof, the antibody or fragment thereof capable of specifically binding to a maize or a soybean aspartate-semialdehyde dehydrogenase enzyme or fragment thereof encoded by a first nucleic acid molecule which specifically hybridizes to a second nucleic acid molecule, the second nucleic acid molecule having a nucleic acid sequence selected from the group consisting of a complement of SEQ ID NO: 901 through SEQ ID NO: 904 and SEQ ID NO: 2649 through SEQ ID NO: 2654 or a substantially purified maize or soybean enzyme aspartate-semialdehyde dehydrogenase or fragment thereof encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 901 through SEQ ID NO: 904 and SEQ ID NO: 2649 through SEQ ID NO: 2654.

The present invention also provides a substantially purified antibody or fragment thereof, the antibody or fragment thereof capable of specifically binding to a maize or a soybean *O*-succinylhomoserine (thiol)-lyase enzyme or fragment thereof encoded by a first nucleic acid molecule which specifically hybridizes to a second nucleic acid molecule, the second nucleic acid molecule having a nucleic acid sequence selected from the group consisting of a complement of SEQ ID NO: 905 through SEQ ID NO: 953 and SEQ ID NO: 2655 through SEQ ID NO: 2660 or a substantially purified maize or soybean *O*-succinylhomoserine (thiol)-lyase enzyme or fragment thereof encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 905 through SEQ ID NO: 953 and SEQ ID NO: 2655 through SEQ ID NO: 2660.

The present invention also provides a substantially purified antibody or fragment thereof, the antibody or fragment thereof capable of specifically binding to a maize or a soybean cystathionine  $\beta$ -lyase enzyme or fragment thereof encoded by a first nucleic acid molecule which specifically hybridizes to a second nucleic acid molecule, the second nucleic acid molecule having a nucleic acid sequence selected from the group consisting of a complement of SEQ ID NO: 954 through SEQ ID NO: 963 and SEQ ID NO: 2661 through SEQ ID NO: 2665 or a substantially purified maize or soybean cystathionine  $\beta$ -lyase enzyme or fragment thereof encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 954 through SEQ ID NO: 963 and SEQ ID NO: 2661 through SEQ ID NO: 2665.

The present invention also provides a substantially purified antibody or fragment thereof, the antibody or fragment thereof capable of specifically binding to a maize or a soybean 5-methyltetrahydropteroyltriglutamate-homocysteine-S-methyltransferase enzyme or fragment thereof encoded by a first nucleic acid molecule which specifically hybridizes to a second nucleic

acid molecule, the second nucleic acid molecule having a nucleic acid sequence selected from the group consisting of a complement of SEQ ID NO: 964 through SEQ ID NO: 1353 and SEQ ID NO: 2666 through SEQ ID NO: 2992 or a substantially purified maize or soybean 5-methyltetrahydropteroyltriglutamate-homocysteine-S-methyltransferase enzyme or fragment thereof encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 964 through SEQ ID NO: 1353 and SEQ ID NO: 2666 through SEQ ID NO: 2992.

The present invention also provides a substantially purified antibody or fragment thereof, the antibody or fragment thereof capable of specifically binding to a maize or a soybean adenosylhomocysteinase enzyme or fragment thereof encoded by a first nucleic acid molecule which specifically hybridizes to a second nucleic acid molecule, the second nucleic acid molecule having a nucleic acid sequence selected from the group consisting of a complement of SEQ ID NO: 1354 through SEQ ID NO: 1630 and SEQ ID NO: 2993 through SEQ ID NO: 3199 or a substantially purified maize or soybean adenosylhomocysteinase enzyme or fragment thereof encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1354 through SEQ ID NO: 1630 and SEQ ID NO: 2993 through SEQ ID NO: 3199.

The present invention also provides a substantially purified antibody or fragment thereof, the antibody or fragment thereof capable of specifically binding to a maize or a soybean cystathionine  $\beta$ -synthase enzyme or fragment thereof encoded by a first nucleic acid molecule which specifically hybridizes to a second nucleic acid molecule, the second nucleic acid molecule having a nucleic acid sequence selected from the group consisting of a complement of SEQ ID NO: 1631 through SEQ ID NO: 1632 or a substantially purified maize or soybean cystathionine  $\beta$ -synthase enzyme or fragment thereof encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1631 through SEQ ID NO: 1632.

The present invention also provides a substantially purified antibody or fragment thereof, the antibody or fragment thereof capable of specifically binding to a maize or a soybean cystathionine  $\gamma$ -lyase enzyme or fragment thereof encoded by a first nucleic acid molecule which specifically hybridizes to a second nucleic acid molecule, the second nucleic acid molecule having a nucleic acid sequence selected from the group consisting of a complement of SEQ ID NO: 1633 through SEQ ID NO: 1634 and SEQ ID NO: 3203 through SEQ ID NO: 3204 or a substantially purified maize or soybean cystathionine  $\gamma$ -lyase enzyme or fragment thereof encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1633 through SEQ ID NO: 1634 and SEQ ID NO: 3203 through SEQ ID NO: 3204.

The present invention also provides a substantially purified antibody or fragment thereof, the antibody or fragment thereof capable of specifically binding to a maize or a soybean *O*-acetylhomoserine enzyme or fragment thereof encoded by a first nucleic acid molecule which specifically hybridizes to a second nucleic acid molecule, the second nucleic acid molecule having a nucleic acid sequence selected from the group consisting of a complement of SEQ ID NO: 3200 through SEQ ID NO: 3202 or a substantially purified maize or soybean *O*-acetylhomoserine enzyme or fragment thereof encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 3200 through SEQ ID NO: 3202.

The present invention also provides a transformed plant having a nucleic acid molecule which comprises: (A) an exogenous promoter region which functions in a plant cell to cause the production of a mRNA molecule; (B) a structural nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of (a) a nucleic acid sequence which encodes for methionine adenosyltransferase or fragment thereof; (b) a nucleic acid sequence which encodes for S-adenosyl-methionine decarboxylase or fragment thereof; (c) a nucleic acid

sequence which encodes for aspartate kinase or fragment thereof; (d) a nucleic acid sequence which encodes for aspartate-semialdehyde dehydrogenase or fragment thereof; (e) a nucleic acid sequence which encodes for cystathionine gamma-synthase or a fragment thereof; (f) a nucleic acid sequence which encodes for cystathionine beta-lyase or a fragment thereof; (g) a nucleic acid sequence which encodes for 5-methyltetrahydropteroyl- triglutamate-homocysteine-S-methyltransferase or a fragment thereof; and (h) a nucleic acid sequence which is complementary to any of the nucleic acid sequences of (a) through (g); and (C) a 3' non-translated sequence that functions in said plant cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of said mRNA molecule.

The present invention also provides a transformed plant having a nucleic acid molecule which comprises: (A) an exogenous promoter region which functions in a plant cell to cause the production of a mRNA molecule; which is linked to (B) a structural nucleic acid molecule, wherein the structural nucleic acid molecule encodes a plant methionine pathway enzyme or fragment thereof, the structural nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 3204 or fragment thereof; which is linked to (C) a 3' non-translated sequence that functions in the plant cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA molecule.

The present invention also provides a transformed plant having a nucleic acid molecule which comprises: (A) an exogenous promoter region which functions in a plant cell to cause the production of a mRNA molecule; which is linked to (B) a structural nucleic acid molecule, wherein the structural nucleic acid molecule is selected from the group consisting of a nucleic acid molecule that encodes a maize or a soybean methionine adenosyltransferase enzyme or

fragment thereof, a nucleic acid molecule that encodes a maize or a soybean S-adenosylmethionine decarboxylase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or soybean aspartate kinase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean aspartate-semialdehyde dehydrogenase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean *O*-succinylhomoserine (thiol)-lyase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean cystathionine  $\beta$ -lyase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean 5-methyltetrahydropteroyltriglutamate-homocysteine-S-methyltransferase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean adenosylhomocysteinase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean cystathionine  $\beta$ -synthase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean cystathionine  $\gamma$ -lyase enzyme or fragment thereof and a nucleic acid molecule that encodes a maize or a soybean *O*-acetylhomoserine (thiol)-lyase enzyme or fragment thereof; which is linked to (C) a 3' non-translated sequence that functions in the plant cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA molecule.

The present invention also provides a transformed plant having a nucleic acid molecule which comprises: (A) an exogenous promoter region which functions in a plant cell to cause the production of a mRNA molecule; which is linked to (B) a transcribed nucleic acid molecule with a transcribed strand and a non-transcribed strand, wherein the transcribed strand is complementary to a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 3204 or fragment thereof; which is

linked to (C) a 3' non-translated sequence that functions in plant cells to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA molecule.

The present invention also provides a transformed plant having a nucleic acid molecule which comprises: (A) an exogenous promoter region which functions in a plant cell to cause the production of a mRNA molecule; which is linked to: (B) a transcribed nucleic acid molecule with a transcribed strand and a non-transcribed strand, wherein a transcribed mRNA of the transcribed strand is complementary to an endogenous mRNA molecule having a nucleic acid sequence selected from the group consisting of an endogenous mRNA molecule that encodes a maize or a soybean methionine adenosyltransferase enzyme or fragment thereof, an endogenous mRNA molecule that encodes a maize or a soybean S-adenosylmethionine decarboxylase enzyme or fragment thereof, an endogenous mRNA molecule that encodes a maize or a soybean aspartate kinase enzyme or fragment thereof, an endogenous mRNA molecule that encodes a maize or a soybean aspartate-semialdehyde dehydrogenase enzyme or fragment thereof, an endogenous mRNA molecule that encodes a maize or a soybean *O*-succinylhomoserine (thiol)-lyase enzyme or fragment thereof, an endogenous mRNA molecule that encodes a maize or a soybean cystathionine  $\beta$ -lyase enzyme or fragment thereof, an endogenous mRNA molecule that encodes a maize or a soybean 5-methyltetrahydropteroyltriglutamate-homocysteine-S-methyltransferase enzyme or fragment thereof, an endogenous mRNA molecule that encodes a maize or a soybean adenosylhomocysteinase enzyme or fragment thereof, an endogenous mRNA molecule that encodes a maize or a soybean cystathionine  $\beta$ -synthase enzyme or fragment thereof, an endogenous mRNA molecule that encodes a maize or a soybean cystathionine  $\gamma$ -lyase enzyme or fragment thereof and an endogenous mRNA molecule that encodes a maize or a soybean *O*-acetylhomoserine (thiol)-lyase enzyme or fragment thereof; which is linked to (C) a 3'

non-translated sequence that functions in the plant cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA molecule.

The present invention also provides a method for determining a level or pattern in a plant cell of an enzyme in a plant metabolic pathway comprising: (A) incubating, under conditions permitting nucleic acid hybridization, a marker nucleic acid molecule, said marker nucleic acid molecule selected from the group of marker nucleic acid molecules which specifically hybridize to a nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 1 through SEQ ID NO: 3204 or compliments thereof, with a complementary nucleic acid molecule obtained from said plant cell or plant tissue, wherein nucleic acid hybridization between said marker nucleic acid molecule and said complementary nucleic acid molecule obtained from said plant cell or plant tissue permits the detection of an mRNA for said enzyme; (B) permitting hybridization between said marker nucleic acid molecule and said complementary nucleic acid molecule obtained from said plant cell or plant tissue; and (C) detecting the level or pattern of said complementary nucleic acid, wherein the detection of said complementary nucleic acid is predictive of the level or pattern of said enzyme in said plant metabolic pathway.

The present invention also provides a method for determining a level or pattern of a plant methionine pathway enzyme in a plant cell or plant tissue comprising: (A) incubating, under conditions permitting nucleic acid hybridization, a marker nucleic acid molecule, the marker nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 3204 or complement thereof or fragment of either, with a complementary nucleic acid molecule obtained from the plant cell or plant tissue, wherein nucleic acid hybridization between the marker nucleic acid molecule and the complementary nucleic acid molecule obtained from the plant cell or plant tissue permits the detection of the



plant methionine pathway enzyme; (B) permitting hybridization between the marker nucleic acid molecule and the complementary nucleic acid molecule obtained from the plant cell or plant tissue; and (C) detecting the level or pattern of the complementary nucleic acid, wherein the detection of the complementary nucleic acid is predictive of the level or pattern of the plant methionine pathway enzyme.

The present invention also provides a method for determining a level or pattern of a plant methionine pathway enzyme in a plant cell or plant tissue comprising: (A) incubating, under conditions permitting nucleic acid hybridization, a marker nucleic acid molecule, the marker nucleic acid molecule comprising a nucleic acid molecule that encodes a maize or a soybean methionine adenosyltransferase enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean S-adenosylmethionine decarboxylase enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean aspartate kinase enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean aspartate-semialdehyde dehydrogenase enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean *O*-succinylhomoserine (thiol)-lyase enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean cystathionine  $\beta$ -lyase enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean 5-methyltetrahydropteroyltriglutamate-homocysteine-S-methyltransferase enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean adenosylhomocysteinase enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean cystathionine  $\beta$ -synthase enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a

soybean cytsathionine  $\gamma$ -lyase enzyme or complement thereof or fragment of either and a nucleic acid molecule that encodes a maize or a soybean *O*-acetylhomoserine (thiol)-lyase enzyme or complement thereof or fragment of either, with a complementary nucleic acid molecule obtained from the plant cell or plant tissue, wherein nucleic acid hybridization between the marker nucleic acid molecule and the complementary nucleic acid molecule obtained from the plant cell or plant tissue permits the detection of the plant methionine pathway enzyme; (B) permitting hybridization between the marker nucleic acid molecule and the complementary nucleic acid molecule obtained from the plant cell or plant tissue; and (C) detecting the level or pattern of the complementary nucleic acid, wherein the detection of the complementary nucleic acid is predictive of the level or pattern of the plant methionine pathway enzyme.

The present invention also provides a method for determining a level or pattern of a plant methionine pathway enzyme in a plant cell or plant tissue under evaluation which comprises assaying the concentration of a molecule, whose concentration is dependent upon the expression of a gene, the gene specifically hybridizes to a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 3204 or complements thereof, in comparison to the concentration of that molecule present in a reference plant cell or a reference plant tissue with a known level or pattern of the plant methionine pathway enzyme, wherein the assayed concentration of the molecule is compared to the assayed concentration of the molecule in the reference plant cell or reference plant tissue with the known level or pattern of the plant methionine pathway enzyme.

The present invention also provides a method for determining a level or pattern of a plant methionine pathway enzyme in a plant cell or plant tissue under evaluation which comprises assaying the concentration of a molecule, whose concentration is dependent upon the expression

of a gene, the gene specifically hybridizes to a nucleic acid molecule selected from the group consisting of a nucleic acid molecule that encodes a maize or a soybean methionine adenosyltransferase enzyme or complement thereof, a nucleic acid molecule that encodes a maize or a soybean S-adenosylmethionine decarboxylase enzyme or complement thereof, a nucleic acid molecule that encodes a maize or a soybean aspartate kinase enzyme or complement thereof, a nucleic acid molecule that encodes a maize or a soybean aspartate-semialdehyde dehydrogenase enzyme or complement thereof, a nucleic acid molecule that encodes a maize or a soybean *O*-succinylhomoserine (thiol)-lyase enzyme or complement thereof, a nucleic acid molecule that encodes a maize or a soybean cystathionine  $\beta$ -lyase enzyme or complement thereof, a nucleic acid molecule that encodes a maize or a soybean 5-methyltetrahydropteroyltriglutamate-homocysteine-S-methyltransferase enzyme or complement thereof, a nucleic acid molecule that encodes a maize or a soybean adenosylhomocysteinease enzyme or complement thereof, a nucleic acid molecule that encodes a maize or a soybean cystathionine  $\beta$ -synthase enzyme or complement thereof, a nucleic acid molecule that encodes a maize or a soybean cystathionine  $\gamma$ -lyase enzyme or complement thereof and a nucleic acid molecule that encodes a maize or a soybean *O*-acetylhomoserine (thiol)-lyase enzyme or complement thereof, in comparison to the concentration of that molecule present in a reference plant cell or a reference plant tissue with a known level or pattern of the plant methionine pathway enzyme, wherein the assayed concentration of the molecule is compared to the assayed concentration of the molecule in the reference plant cell or the reference plant tissue with the known level or pattern of the plant methionine pathway enzyme.

A method of determining a mutation in a plant whose presence is predictive of a mutation affecting a level or pattern of a protein comprising the steps: (A) incubating, under conditions

permitting nucleic acid hybridization, a marker nucleic acid, said marker nucleic acid selected from the group of marker nucleic acid molecules which specifically hybridize to a nucleic acid molecule having a nucleic acid sequence selected from the group of SEQ ID NO: 1 through SEQ ID NO: 3204 or complements thereof and a complementary nucleic acid molecule obtained from said plant, wherein nucleic acid hybridization between said marker nucleic acid molecule and said complementary nucleic acid molecule obtained from said plant permits the detection of a polymorphism whose presence is predictive of a mutation affecting said level or pattern of said plant methionine pathway enzyme in said plant; (B) permitting hybridization between said marker nucleic acid molecule and said complementary nucleic acid molecule obtained from said plant; and (C) detecting the presence of said polymorphism, wherein the detection of said polymorphism is predictive of said mutation.

The present invention also provides a method for determining a mutation in a plant whose presence is predictive of a mutation affecting the level or pattern of a plant methionine pathway enzyme comprising the steps: (A) incubating, under conditions permitting nucleic acid hybridization, a marker nucleic acid molecule, the marker nucleic acid molecule comprising a nucleic acid molecule that is linked to a gene, the gene specifically hybridizes to a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 3204 or complements thereof and a complementary nucleic acid molecule obtained from the plant, wherein nucleic acid hybridization between the marker nucleic acid molecule and the complementary nucleic acid molecule obtained from the plant permits the detection of a polymorphism whose presence is predictive of a mutation affecting the level or pattern of the plant methionine pathway enzyme in the plant; (B) permitting hybridization between the marker nucleic acid molecule and the complementary nucleic acid molecule

obtained from the plant; and (C) detecting the presence of the polymorphism, wherein the detection of the polymorphism is predictive of the mutation.

The present invention also provides a method for determining a mutation in a plant whose presence is predictive of a mutation affecting the level or pattern of a plant methionine pathway enzyme comprising the steps: (A) incubating, under conditions permitting nucleic acid hybridization, a marker nucleic acid molecule, the marker nucleic acid molecule comprising a nucleic acid molecule that is linked to a gene, the gene specifically hybridizes to a nucleic acid molecule selected from the group consisting of a nucleic acid molecule that encodes a maize or a soybean methionine adenosyltransferase enzyme or complement thereof, a nucleic acid molecule that encodes a maize or a soybean S-adenosylmethionine decarboxylase enzyme or complement thereof, a nucleic acid molecule that encodes a maize or a soybean aspartate kinase enzyme or complement thereof, a nucleic acid molecule that encodes a maize or a soybean aspartate-semialdehyde dehydrogenase enzyme or complement thereof, a nucleic acid molecule that encodes a maize or a soybean *O*-succinylhomoserine (thiol)-lyase enzyme or complement thereof, a nucleic acid molecule that encodes a maize or a soybean cystathionine  $\beta$ -lyase enzyme or complement thereof, a nucleic acid molecule that encodes a maize or a soybean 5-methyltetrahydropteroyltriglutamate-homocysteine-S-methyltransferase enzyme or complement thereof, a nucleic acid molecule that encodes a maize or a soybean adenosylhomocysteinase enzyme or complement thereof, a nucleic acid molecule that encodes a maize or a soybean cystathionine  $\beta$ -synthase enzyme or complement thereof, a nucleic acid molecule that encodes a maize or a soybean cystathionine  $\gamma$ -lyase enzyme or complement thereof and a nucleic acid molecule that encodes a maize or a soybean *O*-acetylhomoserine (thiol)-lyase enzyme or complement thereof and a complementary nucleic acid molecule obtained from the plant,

wherein nucleic acid hybridization between the marker nucleic acid molecule and the complementary nucleic acid molecule obtained from the plant permits the detection of a polymorphism whose presence is predictive of a mutation affecting the level or pattern of the plant methionine pathway enzyme in the plant; (B) permitting hybridization between the marker nucleic acid molecule and the complementary nucleic acid molecule obtained from the plant; and (C) detecting the presence of the polymorphism, wherein the detection of the polymorphism is predictive of the mutation.

A method of producing a plant containing an overexpressed protein comprising: (A) transforming said plant with a functional nucleic acid molecule, wherein the functional nucleic acid molecule comprises a promoter region, wherein said promoter region is linked to a structural region, wherein said structural region has a nucleic acid sequence selected from group consisting of SEQ ID NO: 1 through SEQ ID NO: 3204 wherein said structural region is linked to a 3' non-translated sequence that functions in the plant to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and wherein said functional nucleic acid molecule results in overexpression of the protein; and (B) growing said transformed plant.

The present invention also provides a method of producing a plant containing an overexpressed plant methionine enzyme comprising: (A) transforming the plant with a functional nucleic acid molecule, wherein the functional nucleic acid molecule comprises a promoter region, wherein the promoter region is linked to a structural region, wherein the structural region comprises a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 3204 or fragment thereof; wherein the structural region is linked to a 3' non-translated sequence that functions in the plant to cause

termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and wherein the functional nucleic acid molecule results in overexpression of the plant methionine pathway enzyme; and (B) growing the transformed plant.

The present invention also provides a method of producing a plant containing an overexpressed plant methionine pathway enzyme comprising: (A) transforming the plant with a functional nucleic acid molecule, wherein the functional nucleic acid molecule comprises a promoter region, wherein the promoter region is linked to a structural region, wherein the structural region comprises a nucleic acid molecule selected from the group consisting of a nucleic acid molecule that encodes a maize or a soybean methionine adenosyltransferase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean S-adenosylmethionine decarboxylase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean aspartate kinase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean aspartate-semialdehyde dehydrogenase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean *O*-succinylhomoserine (thiol)-lyase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean cystathionine  $\beta$ -lyase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean 5-methyltetrahydropteroyltriglutamate-homocysteine-S-methyltransferase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean adenosylhomocysteinase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean cystathionine  $\beta$ -synthase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean cystathionine  $\gamma$ -lyase enzyme or fragment thereof and a nucleic acid molecule that encodes a maize or a soybean *O*-acetylhomoserine (thiol)-lyase enzyme or fragment thereof; wherein the structural region is

linked to a 3' non-translated sequence that functions in the plant to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and wherein the functional nucleic acid molecule results in overexpression of the plant methionine pathway enzyme protein; and (B) growing the transformed plant.

The present invention also provides a method of producing a plant containing reduced levels of a plant methionine pathway enzyme comprising: (A) transforming the plant with a functional nucleic acid molecule, wherein the functional nucleic acid molecule comprises a promoter region, wherein the promoter region is linked to a structural region, wherein the structural region comprises a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 3204; wherein the structural region is linked to a 3' non-translated sequence that functions in the plant to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and wherein the functional nucleic acid molecule results in co-suppression of the plant methionine pathway enzyme protein; and (B) growing the transformed plant.

The present invention also provides a method of producing a plant containing reduced levels of a plant methionine pathway enzyme comprising: (A) transforming the plant with a functional nucleic acid molecule, wherein the functional nucleic acid molecule comprises a promoter region, wherein the promoter region is linked to a structural region, wherein the structural region comprises a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of a nucleic acid molecule that encodes a maize or a soybean methionine adenosyltransferase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean S-adenosylmethionine decarboxylase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean aspartate kinase enzyme or fragment thereof, a





to an endogenous mRNA molecule; and wherein the transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in the plant cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and (B) growing the transformed plant.

The present invention also provides a method for reducing expression of a plant methionine pathway enzyme in a plant comprising: (A) transforming the plant with a nucleic acid molecule, the nucleic acid molecule having an exogenous promoter region which functions in a plant cell to cause the production of a mRNA molecule, wherein the exogenous promoter region is linked to a transcribed nucleic acid molecule having a transcribed strand and a non-transcribed strand, wherein a transcribed mRNA of the transcribed strand is complementary to a nucleic acid molecule selected from the group consisting of an endogenous mRNA molecule that encodes a maize or a soybean methionine adenosyltransferase enzyme or fragment thereof, an endogenous mRNA molecule that encodes a maize or a soybean S-adenosylmethionine decarboxylase enzyme or fragment thereof, an endogenous mRNA molecule that encodes a maize or a soybean aspartate kinase enzyme or fragment thereof, an endogenous mRNA molecule that encodes a maize or a soybean aspartate-semialdehyde dehydrogenase enzyme or fragment thereof, an endogenous mRNA molecule that encodes a maize or a soybean *O*-succinylhomoserine (thiol)-lyase enzyme or fragment thereof, an endogenous mRNA molecule that encodes a maize or a soybean cystathionine  $\beta$ -lyase enzyme or fragment thereof, an endogenous mRNA molecule that encodes a maize or a soybean 5-methyltetrahydropteroyltriglutamate-homocysteine-S-methyltransferase enzyme or fragment thereof, an endogenous mRNA molecule that encodes a maize or a soybean adenosylhomocysteinase enzyme or fragment thereof, an endogenous mRNA molecule that encodes a maize or a soybean cystathionine  $\beta$ -synthase enzyme or fragment

thereof, an endogenous mRNA molecule that encodes a maize or a soybean cystathionine  $\gamma$ -lyase enzyme or fragment thereof and an endogenous mRNA molecule that encodes a maize or a soybean *O*-acetylhomoserine (thiol)-lyase enzyme or fragment thereof; and wherein the transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in the plant cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and (B) growing the transformed plant.

The present invention also provides a method of determining an association between a polymorphism and a plant trait comprising: (A) hybridizing a nucleic acid molecule specific for the polymorphism to genetic material of a plant, wherein the nucleic acid molecule has a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 3204 or complements thereof or fragment thereof; and (B) calculating the degree of association between the polymorphism and the plant trait.

The present invention also provides a method of determining an association between a polymorphism and a plant trait comprising: (A) hybridizing a nucleic acid molecule specific for the polymorphism to genetic material of a plant, wherein the nucleic acid molecule is selected from the group consisting of a nucleic acid molecule that encodes a maize or a soybean methionine adenosyltransferase enzyme complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean S-adenosylmethionine decarboxylase enzyme complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean aspartate kinase enzyme complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean aspartate-semialdehyde dehydrogenase enzyme complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean *O*-succinylhomoserine (thiol)-lyase enzyme complement thereof or fragment of either, a

nucleic acid molecule that encodes a maize or a soybean cystathionine  $\beta$ -lyase enzyme complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean 5-methyltetrahydropteroyltriglutamate-homocysteine-S-methyltransferase enzyme or complement thereof or fragment of either; a nucleic acid molecule that encodes a maize or a soybean adenosylhomocysteinase enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean cystathionine  $\beta$ -synthase enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean cytsathionine  $\gamma$ -lyase enzyme or complement thereof or fragment of either and a nucleic acid molecule that encodes a maize or a soybean *O*-acetylhomoserine (thiol)-lyase enzyme or complement thereof or fragment of either and (B) calculating the degree of association between the polymorphism and the plant trait.

The present invention also provides a method of isolating a nucleic acid that encodes a plant methionine pathway enzyme or fragment thereof comprising: (A) incubating under conditions permitting nucleic acid hybridization, a first nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 3204 or complements thereof or fragment of either with a complementary second nucleic acid molecule obtained from a plant cell or plant tissue; (B) permitting hybridization between the first nucleic acid molecule and the second nucleic acid molecule obtained from the plant cell or plant tissue; and (C) isolating the second nucleic acid molecule.

The present invention also provides a method of isolating a nucleic acid molecule that encodes a plant methionine pathway enzyme or fragment thereof comprising: (A) incubating under conditions permitting nucleic acid hybridization, a first nucleic acid molecule selected from the group consisting of a nucleic acid molecule that encodes a maize or a soybean

methionine adenosyltransferase enzyme complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean S-adenosylmethionine decarboxylase enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean aspartate kinase enzyme complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean aspartate-semialdehyde dehydrogenase enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean *O*-succinylhomoserine (thiol)-lyase enzyme or complement thereof or fragment of either and a nucleic acid molecule that encodes a maize or a soybean cystathionine  $\beta$ -lyase enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean 5-methyltetrahydropteroyltriglutamate-homocysteine-S-methyltransferase enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean adenosylhomocysteinase enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean cystathionine  $\beta$ -synthase enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean cytsathionine  $\gamma$ -lyase enzyme or complement thereof or fragment of either and a nucleic acid molecule that encodes a maize or a soybean *O*-acetylhomoserine (thiol)-lyase enzyme or complement thereof or fragment of either, with a complementary second nucleic acid molecule obtained from a plant cell or plant tissue; (B) permitting hybridization between the plant methionine pathway nucleic acid molecule and the complementary nucleic acid molecule obtained from the plant cell or plant tissue; and (C) isolating the second nucleic acid molecule.

## **DETAILED DESCRIPTION OF THE INVENTION**

### **Definitions and Agents of the Present Invention**

#### **Definitions:**

As used herein, a methionine pathway enzyme is any enzyme that is associated with the synthesis or degradation of methionine.

As used herein, a methionine synthesis enzyme is any enzyme that is associated with the synthesis of methionine.

As used herein, a methionine degradation enzyme is any enzyme that is associated with the degradation of methionine.

As used herein, methionine adenosyltransferase is any enzyme that catalyzes the conversion of methionine to S-adenosylmethionine.

As used herein, S-adenosylmethionine decarboxylase is any enzyme that catalyzes the reaction that converts S-adenosylmethionine to (5-deoxy-5-adenosyl)(3-aminopropyl) methylsulfonium salt.

As used herein, aspartate kinase is any enzyme that catalyzes the conversion of aspartate to  $\beta$ -aspartyl phosphate.

As used herein, aspartate semialdehyde dehydrogenase is any enzyme that catalyzes the conversion of  $\beta$ -aspartyl phosphate to aspartate-semialdehyde via an NADPH-dependent reaction.

As used herein, *O*-succinylhomoserine (thiol)-lyase refers to any enzyme that catalyzes the conversion of *O*-phosphohomoserine to and cysteine to cystathionine.

As used herein, cystathionine  $\beta$ -lyase is any enzyme that catalyzes the conversion of cystathionine to homocysteine, pyruvate and ammonia.

As used herein, 5-methyltetrahydropterolytriglutamate-homocysteine S-methyltransferase refers to any enzyme which catalyzes the conversion of homocysteine via methylation to methionine.

As used herein, adenosylhomocysteinase refers to any enzyme that catalyzes the ATP-dependent conversion of S-adenosylmethionine (AdoMet) to methylthioadenosine and L-homoserine.

As used herein, cystathionine  $\beta$ -synthase refers to any enzyme that catalyzes the conversion of homocysteine and serine to cystathionine.

As used herein, cystathionine  $\gamma$ -lyase refers to any enzyme that catalyzes the  $\gamma$  cleavage of cystathionine.

As used herein, *O*-acetylhomoserine (thiol)-lyase refers to any enzyme that catalyzes the conversion of *O*-acetylhomoserine and sulfur to homocysteine.

## **Agents**

### **(a) Nucleic Acid Molecules**

Agents of the present invention include plant nucleic acid molecules and more specifically include maize and soybean nucleic acid molecules and more specifically include nucleic acid molecules of the maize genotypes B73 (Illinois Foundation Seeds, Champaign, Illinois U.S.A.), B73 x Mo17 (Illinois Foundation Seeds, Champaign, Illinois U.S.A.), DK604 (Dekalb Genetics, Dekalb, Illinois U.S.A.), H99 (Illinois Foundation Seeds, Champaign, Illinois U.S.A.), RX601 (Asgrow Seed Company, Des Moines, Iowa), Mo17 (Illinois Foundation Seeds, Champaign, Illinois U.S.A.), and soybean types Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa), C1944 (United States Department of Agriculture (USDA) Soybean Germplasm Collection, Urbana, Illinois U.S.A.), Cristalina (USDA Soybean Germplasm Collection, Urbana,

Illinois U.S.A.), FT108 (Monsoy, Brazil), Hartwig (USDA Soybean Germplasm Collection, Urbana, Illinois U.S.A.), BW211S Null (Tohoku University, Morioka, Japan), PI507354 (USDA Soybean Germplasm Collection, Urbana, Illinois U.S.A.), Asgrow A4922 (Asgrow Seed Company, Des Moines, Iowa U.S.A.), PI227687 (USDA Soybean Germplasm Collection, Urbana, Illinois U.S.A.), PI229358 (USDA Soybean Germplasm Collection, Urbana, Illinois U.S.A.) and Asgrow A3237 (Asgrow Seed Company, Des Moines, Iowa U.S.A.).

A subset of the nucleic acid molecules of the present invention includes nucleic acid molecules that are marker molecules. Another subset of the nucleic acid molecules of the present invention include nucleic acid molecules that encode a protein or fragment thereof. Another subset of the nucleic acid molecules of the present invention are EST molecules.

Fragment nucleic acid molecules may encode significant portion(s) of, or indeed most of, these nucleic acid molecules. Alternatively, the fragments may comprise smaller oligonucleotides (having from about 15 to about 250 nucleotide residues and more preferably, about 15 to about 30 nucleotide residues).

As used herein, an agent, be it a naturally occurring molecule or otherwise may be “substantially purified,” if desired, such that one or more molecules that is or may be present in a naturally occurring preparation containing that molecule will have been removed or will be present at a lower concentration than that at which it would normally be found.

The agents of the present invention will preferably be “biologically active” with respect to either a structural attribute, such as the capacity of a nucleic acid to hybridize to another nucleic acid molecule, or the ability of a protein to be bound by an antibody (or to compete with another molecule for such binding). Alternatively, such an attribute may be catalytic and thus involve the capacity of the agent to mediate a chemical reaction or response.



The agents of the present invention may also be recombinant. As used herein, the term recombinant means any agent (e.g. DNA, peptide etc.), that is, or results, however indirect, from human manipulation of a nucleic acid molecule.

It is understood that the agents of the present invention may be labeled with reagents that facilitate detection of the agent (e.g. fluorescent labels, Prober *et al.*, *Science* 238:336-340 (1987); Albarella *et al.*, EP 144914; chemical labels, Sheldon *et al.*, U.S. Patent 4,582,789; Albarella *et al.*, U.S. Patent 4,563,417; modified bases, Miyoshi *et al.*, EP 119448, all of which are hereby incorporated by reference in their entirety).

It is further understood, that the present invention provides recombinant bacterial, mammalian, microbial, insect, fungal and plant cells and viral constructs comprising the agents of the present invention. (See, for example, Uses of the Agents of the Invention, Section (a) Plant Constructs and Plant Transformants; Section (b) Fungal Constructs and Fungal Transformants; Section (c) Mammalian Constructs and Transformed Mammalian Cells; Section (d) Insect Constructs and Transformed Insect Cells; and Section (e) Bacterial Constructs and Transformed Bacterial Cells)

Nucleic acid molecules or fragments thereof of the present invention are capable of specifically hybridizing to other nucleic acid molecules under certain circumstances. As used herein, two nucleic acid molecules are said to be capable of specifically hybridizing to one another if the two molecules are capable of forming an anti-parallel, double-stranded nucleic acid structure. A nucleic acid molecule is said to be the "complement" of another nucleic acid molecule if they exhibit complete complementarity. As used herein, molecules are said to exhibit "complete complementarity" when every nucleotide of one of the molecules is complementary to a nucleotide of the other. Two molecules are said to be "minimally

complementary” if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under at least conventional "low-stringency" conditions.

Similarly, the molecules are said to be “complementary” if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under conventional "high-stringency" conditions. Conventional stringency conditions are described by Sambrook *et al.*, *Molecular Cloning*, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Press, Cold Spring Harbor, New York (1989) and by Haymes *et al.*, *Nucleic Acid Hybridization, A Practical Approach*, IRL Press, Washington, DC (1985), the entirety of which is herein incorporated by reference. Departures from complete complementarity are therefore permissible, as long as such departures do not completely preclude the capacity of the molecules to form a double-stranded structure. Thus, in order for a nucleic acid molecule to serve as a primer or probe it need only be sufficiently complementary in sequence to be able to form a stable double-stranded structure under the particular solvent and salt concentrations employed.

Appropriate stringency conditions which promote DNA hybridization, for example, 6.0 X sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0 X SSC at 50°C, are known to those skilled in the art or can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0 X SSC at 50°C to a high stringency of about 0.2 X SSC at 50°C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22°C, to high stringency conditions at about 65°C. Both temperature and salt may be varied, or either the temperature or the salt concentration may be held constant while the other variable is changed.

In a preferred embodiment, a nucleic acid of the present invention will specifically hybridize to one or more of the nucleic acid molecules set forth in SEQ ID NO: 1 through SEQ ID NO: 3204 or complements thereof under moderately stringent conditions, for example at about 2.0 X SSC and about 65°C.

In a particularly preferred embodiment, a nucleic acid of the present invention will include those nucleic acid molecules that specifically hybridize to one or more of the nucleic acid molecules set forth in SEQ ID NO: 1 through SEQ ID NO: 3204 or complements thereof under high stringency conditions such as 0.2 X SSC and about 65°C.

In one aspect of the present invention, the nucleic acid molecules of the present invention have one or more of the nucleic acid sequences set forth in SEQ ID NO: 1 through SEQ ID NO: 3204 or complements thereof. In another aspect of the present invention, one or more of the nucleic acid molecules of the present invention share between 100% and 90% sequence identity with one or more of the nucleic acid sequences set forth in SEQ ID NO: 1 through SEQ ID NO: 3204 or complements thereof. In a further aspect of the present invention, one or more of the nucleic acid molecules of the present invention share between 100% and 95% sequence identity with one or more of the nucleic acid sequences set forth in SEQ ID NO: 1 through SEQ ID NO: 3204 or complements thereof. In a more preferred aspect of the present invention, one or more of the nucleic acid molecules of the present invention share between 100% and 98% sequence identity with one or more of the nucleic acid sequences set forth in SEQ ID NO: 1 through SEQ ID NO: 3204 or complements thereof. In an even more preferred aspect of the present invention, one or more of the nucleic acid molecules of the present invention share between 100% and 99% sequence identity with one or more of the sequences set forth in SEQ ID NO: 1 through SEQ ID NO: 3204 or complements thereof.

In a further more preferred aspect of the present invention, one or more of the nucleic acid molecules of the present invention exhibit 100% sequence identity with a nucleic acid molecule present within MONN01, SATMON001 through SATMON031, SATMON033, SATMON034, SATMON~001, SATMONN01, SATMONN04 through SATMONN006, CMz029 through CMz031, CMz033, CMz035 through CMz037, CMz039 through CMz042, CMz044 through CMz045, CMz047 through CMz050, SOYMON001 through SOYMON038, Soy51 through Soy56, Soy58 through Soy62, Soy65 through Soy66, Soy 68 through Soy73 and Soy76 through Soy77, Lib9, Lib22 through Lib25, Lib35, Lib80 through Lib81, Lib 144, Lib146, Lib147, Lib190, Lib3032 through Lib3036 and Lib3099 (Monsanto Company, St. Louis, Missouri U.S.A.).

**(i) Nucleic Acid Molecules Encoding Proteins or Fragments Thereof**

Nucleic acid molecules of the present invention can comprise sequences that encode a methionine pathway protein or fragment thereof. Such proteins or fragments thereof include homologues of known proteins in other organisms.

In a preferred embodiment of the present invention, a maize or soybean protein homologue or fragment thereof of the present invention is a homologue of another plant protein. In another preferred embodiment of the present invention, a maize or soybean protein homologue or fragment thereof of the present invention is a homologue of a fungal protein. In another preferred embodiment of the present invention, a maize or soybean protein homologue of the present invention is a homologue of mammalian protein. In another preferred embodiment of the present invention, a maize or soybean protein homologue or fragment thereof of the present invention is a homologue of a bacterial protein. In another preferred embodiment of the present invention, a soybean protein homologue or fragment thereof of the present invention is a

maize protein homologue or fragment thereof of the present invention is a homologue of a soybean protein.

In a preferred embodiment of the present invention, the nucleic molecule of the present invention encodes a maize or soybean homologue protein or fragment thereof where a maize or soybean homologue protein exhibits a BLAST probability score of greater than 1E-12, preferably a BLAST probability score of between about 1E-30 and about 1E-12, even more preferably a BLAST probability score of greater than 1E-30 with its homologue.

In another preferred embodiment of the present invention, the nucleic acid molecule encoding a maize or soybean protein homologue or fragment thereof or fragment thereof exhibits a % identity with its homologue of between about 25% and about 40%, more preferably of between about 40 and about 70%, even more preferably of between about 70% and about 90% and even more preferably between about 90% and 99%. In another preferred embodiment, of the present invention, a maize or soybean protein homologue or fragments thereof exhibits a % identity with its homologue of 100%.

In a preferred embodiment of the present invention, the nucleic molecule of the present invention encodes a maize or soybean homologue protein or fragment thereof where a maize or soybean homologue protein exhibits a BLAST score of greater than 120, preferably a BLAST score of between about 1450 and about 120, even more preferably a BLAST score of greater than 1450 with its homologue.

Nucleic acid molecules of the present invention also include non-maize, non-soybean homologues. Preferred non- homologues are selected from the group consisting of alfalfa, *Arabidopsis*, barley, *Brassica*, broccoli, cabbage, citrus, cotton, garlic, oat, oilseed rape, onion, canola, flax, an ornamental plant, pea, peanut, pepper, potato, rice, rye, sorghum, strawberry,

sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, oil palm and *Phaseolus*.

In a preferred embodiment, nucleic acid molecules having SEQ ID NO: 1 through SEQ ID NO: 3204 or complements and fragments of either can be utilized to obtain such homologues.

The degeneracy of the genetic code, which allows different nucleic acid sequences to code for the same protein or peptide, is known in the literature. (U.S. Patent No. 4,757,006, the entirety of which is herein incorporated by reference).

In an aspect of the present invention, one or more of the nucleic acid molecules of the present invention differ in nucleic acid sequence from those encoding maize or soybean homologue or fragment thereof in SEQ ID NO: 1 through SEQ ID NO: 3204 due to the degeneracy in the genetic code in that they encode the same protein but differ in nucleic acid sequence.

In another further aspect of the present invention, one or more of the nucleic acid molecules of the present invention differ in nucleic acid sequence from those encoding maize or soybean homologue or fragment thereof in SEQ ID NO: 1 through SEQ ID NO: 3204 due to fact that the different nucleic acid sequence encodes a protein having one or more conservative amino acid residue. Examples of conservative substitutions are set forth in Table 1. It is understood that codons capable of coding for such conservative substitutions are known in the art.

**Table 1**

<u>Original Residue</u>	<u>Conservative Substitutions</u>
Ala	Ser
Arg	Lys

0040879-4450

Asn	Gln; His
Asp	Glu
Cys	Ser; Ala
Gln	Asn
Glu	Asp
Gly	Pro
His	Asn; Gln
Ile	Leu; Val
Leu	Ile; Val
Lys	Arg; Gln; Glu
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

In a further aspect of the present invention, one or more of the nucleic acid molecules of the present invention differ in nucleic acid sequence from those encoding a maize or soybean homologue or fragment thereof set forth in SEQ ID NO: 1 through SEQ ID NO: 3204 or fragment thereof due to the fact that one or more codons encoding an amino acid has been

substituted for a codon that encodes a nonessential substitution of the amino acid originally encoded.

Agents of the present invention include nucleic acid molecules that encode a maize or soybean methionine pathway protein or fragment thereof and particularly substantially purified nucleic acid molecules selected from the group consisting of a nucleic acid molecule that encodes a maize or soybean methionine adenosyltransferase protein or fragment thereof, a nucleic acid molecule that encodes a maize or soybean S-adenosylmethionine decarboxylase protein or fragment thereof, a nucleic acid molecule that encodes a maize or soybean aspartate kinase protein or fragment thereof, a nucleic acid molecule that encode a maize or soybean aspartate-semialdehyde dehydrogenase protein or fragment thereof, a nucleic acid molecule that encodes a maize or soybean *O*-succinylhomoserine (thiol)-lyase protein or fragment thereof, a nucleic acid molecule that encodes a maize or soybean cystathionine  $\beta$ -lyase protein or fragment thereof, a nucleic acid molecule that encodes a maize or soybean 5-methyltetrahydropteroyltriglutamate-homocysteine-S-methyltransferase protein or fragment thereof, a nucleic acid molecule that encodes a maize or soybean adenosylhomocysteine protein or fragment thereof, a nucleic acid molecule that encodes a maize or soybean cystathionine  $\beta$ -synthase protein or fragment thereof, a nucleic acid molecule that encodes a maize or soybean cystathionine  $\gamma$ -lyase protein or fragment thereof, and a nucleic acid molecule that encodes a maize or soybean *O*-acetylhomoserine (thiol)-lyase protein or fragment thereof.

Non-limiting examples of such nucleic acid molecules of the present invention are nucleic acid molecules comprising: SEQ ID NO: 1 through SEQ ID NO: 3204 or fragment thereof that encode for a methionine pathway protein or fragment thereof, SEQ ID NO: 1 through SEQ ID NO: 429 and SEQ ID NO: 1635 through SEQ ID NO: 2479 or fragment thereof that



encode for a methionine adenosyltransferase protein or fragment thereof, SEQ ID NO: 430  
 through SEQ ID NO: 857 and SEQ ID NO: 2480 through SEQ ID NO: 2623 or fragment thereof  
 that encode for a S-adenosylmethionine decarboxylase protein or fragment thereof, SEQ ID NO:  
 858 through SEQ ID NO: 900 and SEQ ID NO: 2624 through SEQ ID NO: 2648 or fragment  
 thereof that encode for a aspartate kinase protein or fragment thereof, SEQ ID NO: 901 through  
 SEQ ID NO: 904 and SEQ ID NO: 2649 through SEQ ID NO: 2654 or fragment thereof that  
 encode for a aspartate-semialdehyde dehydrogenase protein or fragment thereof, SEQ ID NO:  
 905 through SEQ ID NO: 953 and SEQ ID NO: 2655 through SEQ ID NO: 2660 or fragment  
 thereof that encode for a *O*-succinylhomoserine (thiol)-lyase protein or fragment thereof, SEQ ID  
 NO: 954 through SEQ ID NO: 963 and SEQ ID NO: 2655 through SEQ ID NO: 2660 or  
 fragment thereof that encode for a cystathionine  $\beta$ -lyase protein or fragment thereof, SEQ ID  
 NO: 964 through SEQ ID NO: 1353 and SEQ ID NO: 2666 through SEQ ID NO: 2992 or  
 fragment thereof that encode for a 5-methyltetrahydropteroyltriglutamate-homocysteine-S-  
 methyltransferase protein or fragment thereof, SEQ ID NO: 1354 through SEQ ID NO: 1630 and  
 SEQ ID NO: 2993 through SEQ ID NO: 3199 or fragment thereof that encode for an  
 adenosylhomocysteinase protein or fragment thereof, SEQ ID NO: 1631 through SEQ ID NO:  
 1632 or fragment thereof that encode for a cystathionine  $\beta$ -synthase protein or fragment thereof,  
 SEQ ID NO: 1633 through SEQ ID NO: 1634 and SEQ ID NO: 3203 through SEQ ID NO: 3204  
 or fragment thereof that encode for a cystathionine  $\gamma$ -lyase protein or fragment thereof, and SEQ  
 ID NO: 3200 through SEQ ID NO: 3202 or fragment thereof that encode for an *O*-  
 acetylhomoserine (thiol)-lyase protein or fragment thereof.

A nucleic acid molecule of the present invention can also encode an homologue of a maize or soybean methionine adenosyltransferase or fragment thereof, a maize or soybean S-adenosylmethionine decarboxylase or fragment thereof, a maize or soybean aspartate kinase or fragment thereof, a maize or soybean aspartate-semialdehyde dehydrogenase or fragment thereof, a maize or soybean *O*-succinylhomoserine (thiol)-lyase or fragment thereof, a maize or soybean cystathionine  $\beta$ -lyase or fragment thereof, a maize or soybean 5-methyltetrahydropteroyltriglutamate-homocysteine-S-methyltransferase or fragment thereof, a maize or soybean adenosylhomocysteinease or fragment thereof, a maize or soybean cystathionine  $\beta$ -synthase or fragment thereof, a maize or soybean cystathionine  $\gamma$ -lyase or fragment thereof or a maize or soybean *O*-acetylhomoserine (thiol)-lyase or fragment thereof. As used herein a homologue protein molecule or fragment thereof is a counterpart protein molecule or fragment thereof in a second species (*e.g.*, maize methionine adenosyltransferase protein is a homologue of *Arabidopsis* ' methionine adenosyltransferase protein).

## **(ii) Nucleic Acid Molecule Markers and Probes**

One aspect of the present invention concerns markers that include nucleic acid molecules SEQ ID NO: 1 through SEQ ID NO: 3204 or complements thereof or fragments of either that can act as markers. Genetic markers of the present invention include “dominant” or “codominant” markers. “Codominant markers” reveal the presence of two or more alleles (two per diploid individual) at a locus. “Dominant markers” reveal the presence of only a single allele per locus. The presence of the dominant marker phenotype (*e.g.*, a band of DNA) is an indication that one allele is present in either the homozygous or heterozygous condition. The absence of the dominant marker phenotype (*e.g.* absence of a DNA band) is merely evidence that “some other”

undefined allele is present. In the case of populations where individuals are predominantly homozygous and loci are predominately dimorphic, dominant and codominant markers can be equally valuable. As populations become more heterozygous and multi-allelic, codominant markers often become more informative of the genotype than dominant markers. Marker molecules can be, for example, capable of detecting polymorphisms such as single nucleotide polymorphisms (SNPs).

SNPs are single base changes in genomic DNA sequence. They occur at greater frequency and are spaced with a greater uniformity throughout a genome than other reported forms of polymorphism. The greater frequency and uniformity of SNPs means that there is greater probability that such a polymorphism will be found near or in a genetic locus of interest than would be the case for other polymorphisms. SNPs are located in protein-coding regions and noncoding regions of a genome. Some of these SNPs may result in defective or variant protein expression (e.g., as a results of mutations or defective splicing). Analysis (genotyping) of characterized SNPs can require only a plus/minus assay rather than a lengthy measurement, permitting easier automation.

SNPs can be characterized using any of a variety of methods. Such methods include the direct or indirect sequencing of the site, the use of restriction enzymes (Botstein *et al.*, *Am. J. Hum. Genet.* 32:314-331 (1980), the entirety of which is herein incorporated reference; Konieczny and Ausubel, *Plant J.* 4:403-410 (1993), the entirety of which is herein incorporated by reference), enzymatic and chemical mismatch assays (Myers *et al.*, *Nature* 313:495-498 (1985), the entirety of which is herein incorporated by reference), allele-specific PCR (Newton *et al.*, *Nucl. Acids Res.* 17:2503-2516 (1989), the entirety of which is herein incorporated by reference; Wu *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 86:2757-2760 (1989), the entirety of which

is herein incorporated by reference), ligase chain reaction (Barany, *Proc. Natl. Acad. Sci. (U.S.A.)* 88:189-193 (1991), the entirety of which is herein incorporated by reference), single-strand conformation polymorphism analysis (Labrune *et al.*, *Am. J. Hum. Genet.* 48: 1115-1120 (1991), the entirety of which is herein incorporated by reference), primer-directed nucleotide incorporation assays (Kuppuswami *et al.*, *Proc. Natl. Acad. Sci. USA* 88:1143-1147 (1991), the entirety of which is herein incorporated by reference), dideoxy fingerprinting (Sarkar *et al.*, *Genomics* 13:441-443 (1992), the entirety of which is herein incorporated by reference), solid-phase ELISA-based oligonucleotide ligation assays (Nikiforov *et al.*, *Nucl. Acids Res.* 22:4167-4175 (1994), the entirety of which is herein incorporated by reference), oligonucleotide fluorescence-quenching assays (Livak *et al.*, *PCR Methods Appl.* 4:357-362 (1995), the entirety of which is herein incorporated by reference), 5'-nuclease allele-specific hybridization TaqMan assay (Livak *et al.*, *Nature Genet.* 9:341-342 (1995), the entirety of which is herein incorporated by reference), template-directed dye-terminator incorporation (TDI) assay (Chen and Kwok, *Nucl. Acids Res.* 25:347-353 (1997), the entirety of which is herein incorporated by reference), allele-specific molecular beacon assay (Tyagi *et al.*, *Nature Biotech.* 16: 49-53 (1998), the entirety of which is herein incorporated by reference), PinPoint assay (Haff and Smirnov, *Genome Res.* 7: 378-388 (1997), the entirety of which is herein incorporated by reference) and dCAPS analysis (Neff *et al.*, *Plant J.* 14:387-392 (1998), the entirety of which is herein incorporated by reference).

Additional markers, such as AFLP markers, RFLP markers and RAPD markers, can be utilized (Walton, *Seed World* 22-29 (July, 1993), the entirety of which is herein incorporated by reference; Burow and Blake, *Molecular Dissection of Complex Traits*, 13-29, Paterson (ed.), CRC Press, New York (1988), the entirety of which is herein incorporated by reference). DNA

markers can be developed from nucleic acid molecules using restriction endonucleases, the PCR and/or DNA sequence information. RFLP markers result from single base changes or insertions/deletions. These codominant markers are highly abundant in plant genomes, have a medium level of polymorphism and are developed by a combination of restriction endonuclease digestion and Southern blotting hybridization. CAPS are similarly developed from restriction nuclease digestion but only of specific PCR products. These markers are also codominant, have a medium level of polymorphism and are highly abundant in the genome. The CAPS result from single base changes and insertions/deletions.

Another marker type, RAPDs, are developed from DNA amplification with random primers and result from single base changes and insertions/deletions in plant genomes. They are dominant markers with a medium level of polymorphisms and are highly abundant. AFLP markers require using the PCR on a subset of restriction fragments from extended adapter primers. These markers are both dominant and codominant are highly abundant in genomes and exhibit a medium level of polymorphism.

SSRs require DNA sequence information. These codominant markers result from repeat length changes, are highly polymorphic and do not exhibit as high a degree of abundance in the genome as CAPS, AFLPs and RAPDs SNPs also require DNA sequence information. These codominant markers result from single base substitutions. They are highly abundant and exhibit a medium of polymorphism (Rafalski *et al.*, In: *Nonmammalian Genomic Analysis*, Birren and Lai (ed.), Academic Press, San Diego, CA, pp. 75-134 (1996), the entirety of which is herein incorporated by reference). It is understood that a nucleic acid molecule of the present invention may be used as a marker.

Seq 2

A PCR probe is a nucleic acid molecule capable of initiating a polymerase activity while in a double-stranded structure to with another nucleic acid. Various methods for determining the structure of PCR probes and PCR techniques exist in the art. Computer generated searches using programs such as Primer3 ([www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi](http://www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi)), STSPipeline ([www-genome.wi.mit.edu/cgi-bin/www-STS\\_Pipeline](http://www-genome.wi.mit.edu/cgi-bin/www-STS_Pipeline)), or GeneUp (Pesole *et al.*, *BioTechniques* 25:112-123 (1998) the entirety of which is herein incorporated by reference), for example, can be used to identify potential PCR primers.

It is understood that a fragment of one or more of the nucleic acid molecules of the present invention may be a probe and specifically a PCR probe.

**(b) Protein and Peptide Molecules**

A class of agents comprises one or more of the protein or fragments thereof or peptide molecules encoded by SEQ ID NO: 1 through SEQ ID NO: 3204 or one or more of the protein or fragment thereof and peptide molecules encoded by other nucleic acid agents of the present invention. As used herein, the term "protein molecule" or "peptide molecule" includes any molecule that comprises five or more amino acids. It is well known in the art that proteins may undergo modification, including post-translational modifications, such as, but not limited to, disulfide bond formation, glycosylation, phosphorylation, or oligomerization. Thus, as used herein, the term "protein molecule" or "peptide molecule" includes any protein molecule that is modified by any biological or non-biological process. The terms "amino acid" and "amino acids" refer to all naturally occurring L-amino acids. This definition is meant to include norleucine, ornithine, homocysteine and homoserine.

Non-limiting examples of the protein or fragment thereof of the present invention include a maize or soybean methionine pathway protein or fragment thereof; a maize or soybean

methionine adenosyltransferase or fragment thereof, a maize or soybean S-adenosylmethionine decarboxylase or fragment thereof, a maize or soybean aspartate kinase or fragment thereof, a maize or soybean aspartate-semialdehyde dehydrogenase or fragment thereof, a maize or soybean *O*-succinylhomoserine (thiol)-lyase or fragment thereof, a maize or soybean cystathionine  $\beta$ -lyase or fragment thereof, a maize or soybean 5-methyltetrahydropteroyltriglutamate-homocysteine-S-methyltransferase or fragment thereof, a maize or soybean adenosylhomocysteinase or fragment thereof, a maize or soybean cystathionine  $\beta$ -synthase or fragment thereof, a maize or soybean cystathionine  $\gamma$ -lyase or fragment thereof or a maize or soybean *O*-acetylhomoserine (thiol)-lyase or fragment thereof.

Non-limiting examples of the protein or fragment molecules of the present invention are an methionine pathway protein or fragment thereof encoded by: SEQ ID NO: 1 through SEQ ID NO: 3204 or fragment thereof that encode for a methionine pathway protein or fragment thereof, SEQ ID NO: 1 through SEQ ID NO: 429 and SEQ ID NO: 1635 through SEQ ID NO: 2479 or fragment thereof that encode for a methionine adenosyltransferase protein or fragment thereof, SEQ ID NO: 430 through SEQ ID NO: 857 and SEQ ID NO: 2480 through SEQ ID NO: 2623 or fragment thereof that encode for a S-adenosylmethionine decarboxylase protein or fragment thereof, SEQ ID NO: 858 through SEQ ID NO: 900 and SEQ ID NO: 2624 through SEQ ID NO: 2648 or fragment thereof that encode for a aspartate kinase protein or fragment thereof, SEQ ID NO: 901 through SEQ ID NO: 904 and SEQ ID NO: 2649 through SEQ ID NO: 2654 or fragment thereof that encode for a aspartate-semialdehyde dehydrogenase protein or fragment thereof, SEQ ID NO: 905 through SEQ ID NO: 953 and SEQ ID NO: 2655 through SEQ ID NO: 2660 or fragment thereof that encode for a *O*-succinylhomoserine (thiol)-lyase protein or

fragment thereof, SEQ ID NO: 954 through SEQ ID NO: 963 and SEQ ID NO: 2655 through SEQ ID NO: 2660 or fragment thereof that encode for a cystathionine  $\beta$ -lyase or fragment thereof, SEQ ID NO: 964 through SEQ ID NO: 1353 and SEQ ID NO: 2666 through SEQ ID NO: 2992 or fragment thereof that encode for a 5-methyltetrahydropteroyltriglutamate-homocysteine-S-methyltransferase protein or fragment thereof, SEQ ID NO: 1354 through SEQ ID NO: 1630 and SEQ ID NO: 2993 through SEQ ID NO: 3199 or fragment thereof that encode for an adenosylhomocysteinase protein or fragment thereof, SEQ ID NO: 1631 through SEQ ID NO: 1632 or fragment thereof that encode for a cystathionine  $\beta$ -synthase protein or fragment thereof, SEQ ID NO: 1633 through SEQ ID NO: 1634 and SEQ ID NO: 3203 through SEQ ID NO: 3204 or fragment thereof that encode for a cystathionine  $\gamma$ -lyase protein or fragment thereof, and SEQ ID NO: 3200 through SEQ ID NO: 3202 or fragment thereof that encode for an *O*-acetylhomoserine (thiol)-lyase protein or fragment thereof.

One or more of the protein or fragment of peptide molecules may be produced via chemical synthesis, or more preferably, by expressing in a suitable bacterial or eucaryotic host. Suitable methods for expression are described by Sambrook *et al.*, (In: *Molecular Cloning, A Laboratory Manual, 2nd Edition, Cold Spring Harbor Press*, Cold Spring Harbor, New York (1989)), or similar texts. For example, the protein may be expressed in, for example, Uses of the Agents of the Invention, Section (a) Plant Constructs and Plant Transformants; Section (b) Fungal Constructs and Fungal Transformants; Section (c) Mammalian Constructs and Transformed Mammalian Cells; Section (d) Insect Constructs and Transformed Insect Cells; and Section (e) Bacterial Constructs and Transformed Bacterial Cells.



A “protein fragment” is a peptide or polypeptide molecule whose amino acid sequence comprises a subset of the amino acid sequence of that protein. A protein or fragment thereof that comprises one or more additional peptide regions not derived from that protein is a “fusion” protein. Such molecules may be derivatized to contain carbohydrate or other moieties (such as keyhole limpet hemocyanin, etc.). Fusion protein or peptide molecules of the present invention are preferably produced via recombinant means.

Another class of agents comprise protein or peptide molecules or fragments or fusions thereof encoded by SEQ ID NO: 1 through SEQ ID NO: 3204 or complements thereof in which conservative, non-essential or non-relevant amino acid residues have been added, replaced or deleted. Computerized means for designing modifications in protein structure are known in the art (Dahiyat and Mayo, *Science* 278:82-87 (1997), the entirety of which is herein incorporated by reference).

The protein molecules of the present invention include plant homologue proteins. An example of such a homologue is a homologue protein of a non-maize or non soybean plant species, that include but not limited to alfalfa, *Arabidopsis*, barley, *Brassica*, broccoli, cabbage, citrus, cotton, garlic, oat, oilseed rape, onion, canola, flax, an ornamental plant, pea, peanut, pepper, potato, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, oil palm, *Phaseolus* etc. Particularly preferred non-maize or non-soybean for use for the isolation of homologs would include, *Arabidopsis*, barley, cotton, oat, oilseed rape, rice, canola, ornamentals, sugarcane, sugarbeet, tomato, potato, wheat and turf grasses. Such a homologue can be obtained by any of a variety of methods. Most preferably, as indicated above, one or more of the disclosed sequences (SEQ ID NO: 1 through SEQ ID NO: 3204 or complements thereof) will be

used to define a pair of primers that may be used to isolate the homologue-encoding nucleic acid molecules from any desired species. Such molecules can be expressed to yield homologues by recombinant means.

### **(c) Antibodies**

One aspect of the present invention concerns antibodies, single-chain antigen binding molecules, or other proteins that specifically bind to one or more of the protein or peptide molecules of the present invention and their homologues, fusions or fragments. Such antibodies may be used to quantitatively or qualitatively detect the protein or peptide molecules of the present invention. As used herein, an antibody or peptide is said to “specifically bind” to a protein or peptide molecule of the present invention if such binding is not competitively inhibited by the presence of non-related molecules.

Nucleic acid molecules that encode all or part of the protein of the present invention can be expressed, via recombinant means, to yield protein or peptides that can in turn be used to elicit antibodies that are capable of binding the expressed protein or peptide. Such antibodies may be used in immunoassays for that protein. Such protein-encoding molecules, or their fragments may be a “fusion” molecule (i.e., a part of a larger nucleic acid molecule) such that, upon expression, a fusion protein is produced. It is understood that any of the nucleic acid molecules of the present invention may be expressed, via recombinant means, to yield proteins or peptides encoded by these nucleic acid molecules.

The antibodies that specifically bind proteins and protein fragments of the present invention may be polyclonal or monoclonal and may comprise intact immunoglobulins, or antigen binding portions of immunoglobulins fragments (such as  $F(ab')$ ,  $F(ab')_2$ ), or single-chain

immunoglobulins producible, for example, via recombinant means. It is understood that practitioners are familiar with the standard resource materials which describe specific conditions and procedures for the construction, manipulation and isolation of antibodies (*see*, for example, Harlow and Lane, In: *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1988), the entirety of which is herein incorporated by reference).

Murine monoclonal antibodies are particularly preferred. BALB/c mice are preferred for this purpose, however, equivalent strains may also be used. The animals are preferably immunized with approximately 25 µg of purified protein (or fragment thereof) that has been emulsified in a suitable adjuvant (such as TiterMax adjuvant (Vaxcel, Norcross, GA)). Immunization is preferably conducted at two intramuscular sites, one intraperitoneal site and one subcutaneous site at the base of the tail. An additional i.v. injection of approximately 25 µg of antigen is preferably given in normal saline three weeks later. After approximately 11 days following the second injection, the mice may be bled and the blood screened for the presence of anti-protein or peptide antibodies. Preferably, a direct binding Enzyme-Linked Immunoassay (ELISA) is employed for this purpose.

More preferably, the mouse having the highest antibody titer is given a third i.v. injection of approximately 25 µg of the same protein or fragment. The splenic leukocytes from this animal may be recovered 3 days later and then permitted to fuse, most preferably, using polyethylene glycol, with cells of a suitable myeloma cell line (such as, for example, the P3X63Ag8.653 myeloma cell line). Hybridoma cells are selected by culturing the cells under “HAT” (hypoxanthine-aminopterin-thymine) selection for about one week. The resulting clones may then be screened for their capacity to produce monoclonal antibodies (“mAbs”), preferably by direct ELISA.

In one embodiment, anti-protein or peptide monoclonal antibodies are isolated using a fusion of a protein or peptide of the present invention, or conjugate of a protein or peptide of the present invention, as immunogens. Thus, for example, a group of mice can be immunized using a fusion protein emulsified in Freund's complete adjuvant (*e.g.* approximately 50 µg of antigen per immunization). At three week intervals, an identical amount of antigen is emulsified in Freund's incomplete adjuvant and used to immunize the animals. Ten days following the third immunization, serum samples are taken and evaluated for the presence of antibody. If antibody titers are too low, a fourth booster can be employed. Polysera capable of binding the protein or peptide can also be obtained using this method.

In a preferred procedure for obtaining monoclonal antibodies, the spleens of the above-described immunized mice are removed, disrupted and immune splenocytes are isolated over a ficoll gradient. The isolated splenocytes are fused, using polyethylene glycol with BALB/c-derived HGPRT (hypoxanthine guanine phosphoribosyl transferase) deficient P3x63xAg8.653 plasmacytoma cells. The fused cells are plated into 96 well microtiter plates and screened for hybridoma fusion cells by their capacity to grow in culture medium supplemented with hypoxanthine, aminopterin and thymidine for approximately 2-3 weeks.

Hybridoma cells that arise from such incubation are preferably screened for their capacity to produce an immunoglobulin that binds to a protein of interest. An indirect ELISA may be used for this purpose. In brief, the supernatants of hybridomas are incubated in microtiter wells that contain immobilized protein. After washing, the titer of bound immunoglobulin can be determined using, for example, a goat anti-mouse antibody conjugated to horseradish peroxidase. After additional washing, the amount of immobilized enzyme is determined (for example through the use of a chromogenic substrate). Such screening is performed as quickly as possible

after the identification of the hybridoma in order to ensure that a desired clone is not overgrown by non-secreting neighbor cells. Desirably, the fusion plates are screened several times since the rates of hybridoma growth vary. In a preferred sub-embodiment, a different antigenic form may be used to screen the hybridoma. Thus, for example, the splenocytes may be immunized with one immunogen, but the resulting hybridomas can be screened using a different immunogen. It is understood that any of the protein or peptide molecules of the present invention may be used to raise antibodies.

As discussed below, such antibody molecules or their fragments may be used for diagnostic purposes. Where the antibodies are intended for diagnostic purposes, it may be desirable to derivatize them, for example with a ligand group (such as biotin) or a detectable marker group (such as a fluorescent group, a radioisotope or an enzyme).

The ability to produce antibodies that bind the protein or peptide molecules of the present invention permits the identification of mimetic compounds of those molecules. A "mimetic compound" is a compound that is not that compound, or a fragment of that compound, but which nonetheless exhibits an ability to specifically bind to antibodies directed against that compound.

It is understood that any of the agents of the present invention can be substantially purified and/or be biologically active and/or recombinant.

### **Uses of the Agents of the Invention**

Nucleic acid molecules and fragments thereof of the present invention may be employed to obtain other nucleic acid molecules from the same species (e.g., ESTs or fragment thereof from maize may be utilized to obtain other nucleic acid molecules from maize). Such nucleic acid molecules include the nucleic acid molecules that encode the complete coding sequence of a protein and promoters and flanking sequences of such molecules. In addition, such nucleic acid

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molecules include nucleic acid molecules that encode for other isozymes or gene family members. Such molecules can be readily obtained by using the above-described nucleic acid molecules or fragments thereof to screen cDNA or genomic libraries obtained from maize or soybean. Methods for forming such libraries are well known in the art.

Nucleic acid molecules and fragments thereof of the present invention may also be employed to obtain nucleic acid homologues. Such homologues include the nucleic acid molecule of other plants or other organisms (*e.g.*, alfalfa, *Arabidopsis*, barley, *Brassica*, broccoli, cabbage, citrus, cotton, garlic, oat, oilseed rape, onion, canola, flax, an ornamental plant, pea, peanut, pepper, potato, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, oil palm, *Phaseolus*, etc.) including the nucleic acid molecules that encode, in whole or in part, protein homologues of other plant species or other organisms, sequences of genetic elements such as promoters and transcriptional regulatory elements. Such molecules can be readily obtained by using the above-described nucleic acid molecules or fragments thereof to screen cDNA or genomic libraries obtained from such plant species. Methods for forming such libraries are well known in the art. Such homologue molecules may differ in their nucleotide sequences from those found in one or more of SEQ ID NO: 1 through SEQ ID NO: 3204 or complements thereof because complete complementarity is not needed for stable hybridization. The nucleic acid molecules of the present invention therefore also include molecules that, although capable of specifically hybridizing with the nucleic acid molecules may lack "complete complementarity."

Any of a variety of methods may be used to obtain one or more of the above-described nucleic acid molecules (Zamechik *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 83:4143-4146 (1986), the entirety of which is herein incorporated by reference; Goodchild *et al.*, *Proc. Natl. Acad. Sci.*

(U.S.A.) 85:5507-5511 (1988), the entirety of which is herein incorporated by reference; Wickstrom *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 85:1028-1032 (1988), the entirety of which is herein incorporated by reference; Holt *et al.*, *Molec. Cell. Biol.* 8:963-973 (1988), the entirety of which is herein incorporated by reference; Gerwitz *et al.*, *Science* 242:1303-1306 (1988), the entirety of which is herein incorporated by reference; Anfossi *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 86:3379-3383 (1989), the entirety of which is herein incorporated by reference; Becker *et al.*, *EMBO J.* 8:3685-3691 (1989); the entirety of which is herein incorporated by reference). Automated nucleic acid synthesizers may be employed for this purpose. In lieu of such synthesis, the disclosed nucleic acid molecules may be used to define a pair of primers that can be used with the polymerase chain reaction (Mullis *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* 51:263-273 (1986); Erlich *et al.*, European Patent 50,424; European Patent 84,796; European Patent 258,017; European Patent 237,362; Mullis, European Patent 201,184; Mullis *et al.*, U.S. Patent 4,683,202; Erlich, U.S. Patent 4,582,788; and Saiki *et al.*, U.S. Patent 4,683,194, all of which are herein incorporated by reference in their entirety) to amplify and obtain any desired nucleic acid molecule or fragment.

Promoter sequence(s) and other genetic elements, including but not limited to transcriptional regulatory flanking sequences, associated with one or more of the disclosed nucleic acid sequences can also be obtained using the disclosed nucleic acid sequence provided herein. In one embodiment, such sequences are obtained by incubating EST nucleic acid molecules or preferably fragments thereof with members of genomic libraries (*e.g.* maize and soybean) and recovering clones that hybridize to the EST nucleic acid molecule or fragment thereof. In a second embodiment, methods of "chromosome walking," or inverse PCR may be used to obtain such sequences (Frohman *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 85:8998-9002

(1988); Ohara *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 86:5673-5677 (1989); Pang *et al.*, *Biotechniques* 22:1046-1048 (1977); Huang *et al.*, *Methods Mol. Biol.* 69:89-96 (1997); Huang *et al.*, *Method Mol. Biol.* 67:287-294 (1997); Benkel *et al.*, *Genet. Anal.* 13:123-127 (1996); Hartl *et al.*, *Methods Mol. Biol.* 58:293-301 (1996), all of which are herein incorporated by reference in their entirety).

The nucleic acid molecules of the present invention may be used to isolate promoters of cell enhanced, cell specific, tissue enhanced, tissue specific, developmentally or environmentally regulated expression profiles. Isolation and functional analysis of the 5' flanking promoter sequences of these genes from genomic libraries, for example, using genomic screening methods and PCR techniques would result in the isolation of useful promoters and transcriptional regulatory elements. These methods are known to those of skill in the art and have been described (See, for example, Birren *et al.*, *Genome Analysis: Analyzing DNA*, 1, (1997), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., the entirety of which is herein incorporated by reference). Promoters obtained utilizing the nucleic acid molecules of the present invention could also be modified to affect their control characteristics. Examples of such modifications would include but are not limited to enhanced sequences as reported in Uses of the Agents of the Invention, Section (a) Plant Constructs and Plant Transformants. Such genetic elements could be used to enhance gene expression of new and existing traits for crop improvements.

In one sub-aspect, such an analysis is conducted by determining the presence and/or identity of polymorphism(s) by one or more of the nucleic acid molecules of the present invention and more preferably one or more of the EST nucleic acid molecule or fragment thereof which are associated with a phenotype, or a predisposition to that phenotype.



Any of a variety of molecules can be used to identify such polymorphism(s). In one embodiment, one or more of the EST nucleic acid molecules (or a sub-fragment thereof) may be employed as a marker nucleic acid molecule to identify such polymorphism(s). Alternatively, such polymorphisms can be detected through the use of a marker nucleic acid molecule or a marker protein that is genetically linked to (i.e., a polynucleotide that co-segregates with) such polymorphism(s).

In an alternative embodiment, such polymorphisms can be detected through the use of a marker nucleic acid molecule that is physically linked to such polymorphism(s). For this purpose, marker nucleic acid molecules comprising a nucleotide sequence of a polynucleotide located within 1mb of the polymorphism(s) and more preferably within 100kb of the polymorphism(s) and most preferably within 10kb of the polymorphism(s) can be employed.

The genomes of animals and plants naturally undergo spontaneous mutation in the course of their continuing evolution (Gusella, *Ann. Rev. Biochem.* 55:831-854 (1986)). A “polymorphism” is a variation or difference in the sequence of the gene or its flanking regions that arises in some of the members of a species. The variant sequence and the “original” sequence co-exist in the species’ population. In some instances, such co-existence is in stable or quasi-stable equilibrium.

A polymorphism is thus said to be “allelic,” in that, due to the existence of the polymorphism, some members of a species may have the original sequence (i.e., the original “allele”) whereas other members may have the variant sequence (i.e., the variant “allele”). In the simplest case, only one variant sequence may exist and the polymorphism is thus said to be di-allelic. In other cases, the species’ population may contain multiple alleles and the polymorphism is termed tri-allelic, etc. A single gene may have multiple different unrelated

polymorphisms. For example, it may have a di-allelic polymorphism at one site and a multi-allelic polymorphism at another site.

The variation that defines the polymorphism may range from a single nucleotide variation to the insertion or deletion of extended regions within a gene. In some cases, the DNA sequence variations are in regions of the genome that are characterized by short tandem repeats (STRs) that include tandem di- or tri-nucleotide repeated motifs of nucleotides. Polymorphisms characterized by such tandem repeats are referred to as "variable number tandem repeat" ("VNTR") polymorphisms. VNTRs have been used in identity analysis (Weber, U.S. Patent 5,075,217; Armour *et al.*, *FEBS Lett.* 307:113-115 (1992); Jones *et al.*, *Eur. J. Haematol.* 39:144-147 (1987); Horn *et al.*, PCT Patent Application WO91/14003; Jeffreys, European Patent Application 370,719; Jeffreys, U.S. Patent 5,175,082; Jeffreys *et al.*, *Amer. J. Hum. Genet.* 39:11-24 (1986); Jeffreys *et al.*, *Nature* 316:76-79 (1985); Gray *et al.*, *Proc. R. Acad. Soc. Lond.* 243:241-253 (1991); Moore *et al.*, *Genomics* 10:654-660 (1991); Jeffreys *et al.*, *Anim. Genet.* 18:1-15 (1987); Hillel *et al.*, *Anim. Genet.* 20:145-155 (1989); Hillel *et al.*, *Genet.* 124:783-789 (1990), all of which are herein incorporated by reference in their entirety).

The detection of polymorphic sites in a sample of DNA may be facilitated through the use of nucleic acid amplification methods. Such methods specifically increase the concentration of polynucleotides that span the polymorphic site, or include that site and sequences located either distal or proximal to it. Such amplified molecules can be readily detected by gel electrophoresis or other means.

The most preferred method of achieving such amplification employs the polymerase chain reaction ("PCR") (Mullis *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* 51:263-273 (1986); Erlich *et al.*, European Patent Appln. 50,424; European Patent Appln. 84,796; European

Patent Application 258,017; European Patent Appln. 237,362; Mullis, European Patent Appln. 201,184; Mullis *et al.*, U.S. Patent No. 4,683,202; Erlich, U.S. Patent No. 4,582,788; and Saiki *et al.*, U.S. Patent No. 4,683,194), using primer pairs that are capable of hybridizing to the proximal sequences that define a polymorphism in its double-stranded form.

In lieu of PCR, alternative methods, such as the "Ligase Chain Reaction" ("LCR") may be used (Barany, *Proc. Natl. Acad. Sci. (U.S.A.)* 88:189-193 (1991), the entirety of which is herein incorporated by reference). LCR uses two pairs of oligonucleotide probes to exponentially amplify a specific target. The sequences of each pair of oligonucleotides is selected to permit the pair to hybridize to abutting sequences of the same strand of the target. Such hybridization forms a substrate for a template-dependent ligase. As with PCR, the resulting products thus serve as a template in subsequent cycles and an exponential amplification of the desired sequence is obtained.

LCR can be performed with oligonucleotides having the proximal and distal sequences of the same strand of a polymorphic site. In one embodiment, either oligonucleotide will be designed to include the actual polymorphic site of the polymorphism. In such an embodiment, the reaction conditions are selected such that the oligonucleotides can be ligated together only if the target molecule either contains or lacks the specific nucleotide that is complementary to the polymorphic site present on the oligonucleotide. Alternatively, the oligonucleotides may be selected such that they do not include the polymorphic site (see, Segev, PCT Application WO 90/01069, the entirety of which is herein incorporated by reference).

The "Oligonucleotide Ligation Assay" ("OLA") may alternatively be employed (Landegren *et al.*, *Science* 241:1077-1080 (1988), the entirety of which is herein incorporated by reference). The OLA protocol uses two oligonucleotides which are designed to be capable of

hybridizing to abutting sequences of a single strand of a target. OLA, like LCR, is particularly suited for the detection of point mutations. Unlike LCR, however, OLA results in "linear" rather than exponential amplification of the target sequence.

Nickerson *et al.*, have described a nucleic acid detection assay that combines attributes of PCR and OLA (Nickerson *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 87:8923-8927 (1990), the entirety of which is herein incorporated by reference). In this method, PCR is used to achieve the exponential amplification of target DNA, which is then detected using OLA. In addition to requiring multiple and separate, processing steps, one problem associated with such combinations is that they inherit all of the problems associated with PCR and OLA.

Schemes based on ligation of two (or more) oligonucleotides in the presence of nucleic acid having the sequence of the resulting "di-oligonucleotide", thereby amplifying the di-oligonucleotide, are also known (Wu *et al.*, *Genomics* 4:560-569 (1989), the entirety of which is herein incorporated by reference) and may be readily adapted to the purposes of the present invention.

Other known nucleic acid amplification procedures, such as allele-specific oligomers, branched DNA technology, transcription-based amplification systems, or isothermal amplification methods may also be used to amplify and analyze such polymorphisms (Malek *et al.*, U.S. Patent 5,130,238; Davey *et al.*, European Patent Application 329,822; Schuster *et al.*, U.S. Patent 5,169,766; Miller *et al.*, PCT Patent Application WO 89/06700; Kwoh *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 86:1173-1177 (1989); Gingeras *et al.*, PCT Patent Application WO 88/10315; Walker *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 89:392-396 (1992), all of which are herein incorporated by reference in their entirety).

The identification of a polymorphism can be determined in a variety of ways. By correlating the presence or absence of it in a plant with the presence or absence of a phenotype, it is possible to predict the phenotype of that plant. If a polymorphism creates or destroys a restriction endonuclease cleavage site, or if it results in the loss or insertion of DNA (e.g., a VNTR polymorphism), it will alter the size or profile of the DNA fragments that are generated by digestion with that restriction endonuclease. As such, individuals that possess a variant sequence can be distinguished from those having the original sequence by restriction fragment analysis. Polymorphisms that can be identified in this manner are termed "restriction fragment length polymorphisms" ("RFLPs"). RFLPs have been widely used in human and plant genetic analyses (Glassberg, UK Patent Application 2135774; Skolnick *et al.*, *Cytogen. Cell Genet.* 32:58-67 (1982); Botstein *et al.*, *Ann. J. Hum. Genet.* 32:314-331 (1980); Fischer *et al.*, (PCT Application WO90/13668); Uhlen, PCT Application WO90/11369).

Polymorphisms can also be identified by Single Strand Conformation Polymorphism (SSCP) analysis. SSCP is a method capable of identifying most sequence variations in a single strand of DNA, typically between 150 and 250 nucleotides in length (Elles, *Methods in Molecular Medicine: Molecular Diagnosis of Genetic Diseases*, Humana Press (1996), the entirety of which is herein incorporated by reference); Orita *et al.*, *Genomics* 5:874-879 (1989), the entirety of which is herein incorporated by reference). Under denaturing conditions a single strand of DNA will adopt a conformation that is uniquely dependent on its sequence conformation. This conformation usually will be different, even if only a single base is changed. Most conformations have been reported to alter the physical configuration or size sufficiently to be detectable by electrophoresis. A number of protocols have been described for SSCP including, but not limited to, Lee *et al.*, *Anal. Biochem.* 205:289-293 (1992), the entirety of

which is herein incorporated by reference; Suzuki *et al.*, *Anal. Biochem.* 192:82-84 (1991), the entirety of which is herein incorporated by reference; Lo *et al.*, *Nucleic Acids Research* 20:1005-1009 (1992), the entirety of which is herein incorporated by reference; Sarkar *et al.*, *Genomics* 13:441-443 (1992), the entirety of which is herein incorporated by reference. It is understood that one or more of the nucleic acids of the present invention, may be utilized as markers or probes to detect polymorphisms by SSCP analysis.

Polymorphisms may also be found using a DNA fingerprinting technique called amplified fragment length polymorphism (AFLP), which is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA to profile that DNA (Vos *et al.*, *Nucleic Acids Res.* 23:4407-4414 (1995), the entirety of which is herein incorporated by reference). This method allows for the specific co-amplification of high numbers of restriction fragments, which can be visualized by PCR without knowledge of the nucleic acid sequence.

AFLP employs basically three steps. Initially, a sample of genomic DNA is cut with restriction enzymes and oligonucleotide adapters are ligated to the restriction fragments of the DNA. The restriction fragments are then amplified using PCR by using the adapter and restriction sequence as target sites for primer annealing. The selective amplification is achieved by the use of primers that extend into the restriction fragments, amplifying only those fragments in which the primer extensions match the nucleotide flanking the restriction sites. These amplified fragments are then visualized on a denaturing polyacrylamide gel.

AFLP analysis has been performed on *Salix* (Beismann *et al.*, *Mol. Ecol.* 6:989-993 (1997), the entirety of which is herein incorporated by reference), *Acinetobacter* (Janssen *et al.*, *Int. J. Syst. Bacteriol.* 47:1179-1187 (1997), the entirety of which is herein incorporated by

reference), *Aeromonas popoffi* (Huys *et al.*, *Int. J. Syst. Bacteriol.* 47:1165-1171 (1997), the entirety of which is herein incorporated by reference), rice (McCouch *et al.*, *Plant Mol. Biol.* 35:89-99 (1997), the entirety of which is herein incorporated by reference; Nandi *et al.*, *Mol. Gen. Genet.* 255:1-8 (1997), the entirety of which is herein incorporated by reference; Cho *et al.*, *Genome* 39:373-378 (1996), the entirety of which is herein incorporated by reference), barley (*Hordeum vulgare*)(Simons *et al.*, *Genomics* 44:61-70 (1997), the entirety of which is herein incorporated by reference; Waugh *et al.*, *Mol. Gen. Genet.* 255:311-321 (1997), the entirety of which is herein incorporated by reference; Qi *et al.*, *Mol. Gen. Genet.* 254:330-336 (1997), the entirety of which is herein incorporated by reference; Becker *et al.*, *Mol. Gen. Genet.* 249:65-73 (1995), the entirety of which is herein incorporated by reference), potato (Van der Voort *et al.*, *Mol. Gen. Genet.* 255:438-447 (1997), the entirety of which is herein incorporated by reference; Meksem *et al.*, *Mol. Gen. Genet.* 249:74-81 (1995), the entirety of which is herein incorporated by reference), *Phytophthora infestans* (Van der Lee *et al.*, *Fungal Genet. Biol.* 21:278-291 (1997), the entirety of which is herein incorporated by reference), *Bacillus anthracis* (Keim *et al.*, *J. Bacteriol.* 179:818-824 (1997), the entirety of which is herein incorporated by reference), *Astragalus cremnophylax* (Travis *et al.*, *Mol. Ecol.* 5:735-745 (1996), the entirety of which is herein incorporated by reference), *Arabidopsis* (Cnops *et al.*, *Mol. Gen. Genet.* 253:32-41 (1996), the entirety of which is herein incorporated by reference), *Escherichia coli* (Lin *et al.*, *Nucleic Acids Res.* 24:3649-3650 (1996), the entirety of which is herein incorporated by reference), *Aeromonas* (Huys *et al.*, *Int. J. Syst. Bacteriol.* 46:572-580 (1996), the entirety of which is herein incorporated by reference), nematode (Folkertsma *et al.*, *Mol. Plant Microbe Interact.* 9:47-54 (1996), the entirety of which is herein incorporated by reference), tomato (Thomas *et al.*, *Plant J.* 8:785-794 (1995), the entirety of which is herein incorporated by reference) and human (Latorra

*et al.*, *PCR Methods Appl.* 3:351-358 (1994), the entirety of which is herein incorporated by reference). AFLP analysis has also been used for fingerprinting mRNA (Money *et al.*, *Nucleic Acids Res.* 24:2616-2617 (1996), the entirety of which is herein incorporated by reference; Bachem *et al.*, *Plant J.* 9:745-753 (1996), the entirety of which is herein incorporated by reference). It is understood that one or more of the nucleic acids of the present invention, may be utilized as markers or probes to detect polymorphisms by AFLP analysis or for fingerprinting RNA.

Polymorphisms may also be found using random amplified polymorphic DNA (RAPD) (Williams *et al.*, *Nucl. Acids Res.* 18:6531-6535 (1990), the entirety of which is herein incorporated by reference) and cleaveable amplified polymorphic sequences (CAPS) (Lyamichev *et al.*, *Science* 260:778-783 (1993), the entirety of which is herein incorporated by reference). It is understood that one or more of the nucleic acid molecules of the present invention, may be utilized as markers or probes to detect polymorphisms by RAPD or CAPS analysis.

Through genetic mapping, a fine scale linkage map can be developed using DNA markers and, then, a genomic DNA library of large-sized fragments can be screened with molecular markers linked to the desired trait. Molecular markers are advantageous for agronomic traits that are otherwise difficult to tag, such as resistance to pathogens, insects and nematodes, tolerance to abiotic stress, quality parameters and quantitative traits such as high yield potential.

The essential requirements for marker-assisted selection in a plant breeding program are: (1) the marker(s) should co-segregate or be closely linked with the desired trait; (2) an efficient means of screening large populations for the molecular marker(s) should be available; and (3) the screening technique should have high reproducibility across laboratories and preferably be economical to use and be user-friendly.



The genetic linkage of marker molecules can be established by a gene mapping model such as, without limitation, the flanking marker model reported by Lander and Botstein, *Genetics* 121:185-199 (1989) and the interval mapping, based on maximum likelihood methods described by Lander and Botstein, *Genetics* 121:185-199 (1989) and implemented in the software package MAPMAKER/QTL (Lincoln and Lander, *Mapping Genes Controlling Quantitative Traits Using MAPMAKER/QTL*, Whitehead Institute for Biomedical Research, Massachusetts, (1990). Additional software includes Qgene, Version 2.23 (1996), Department of Plant Breeding and Biometry, 266 Emerson Hall, Cornell University, Ithaca, NY, the manual of which is herein incorporated by reference in its entirety). Use of Qgene software is a particularly preferred approach.

A maximum likelihood estimate (MLE) for the presence of a marker is calculated, together with an MLE assuming no QTL effect, to avoid false positives. A  $\log_{10}$  of an odds ratio (LOD) is then calculated as:  $LOD = \log_{10}(\text{MLE for the presence of a QTL} / \text{MLE given no linked QTL})$ .

The LOD score essentially indicates how much more likely the data are to have arisen assuming the presence of a QTL than in its absence. The LOD threshold value for avoiding a false positive with a given confidence, say 95%, depends on the number of markers and the length of the genome. Graphs indicating LOD thresholds are set forth in Lander and Botstein, *Genetics* 121:185-199 (1989) the entirety of which is herein incorporated by reference and further described by Arús and Moreno-González, *Plant Breeding*, Hayward *et al.*, (eds.) Chapman & Hall, London, pp. 314-331 (1993), the entirety of which is herein incorporated by reference.

Additional models can be used. Many modifications and alternative approaches to interval mapping have been reported, including the use non-parametric methods (Kruglyak and Lander, *Genetics* 139:1421-1428 (1995), the entirety of which is herein incorporated by reference). Multiple regression methods or models can be also be used, in which the trait is regressed on a large number of markers (Jansen, *Biometrics in Plant Breeding*, van Oijen and Jansen (eds.), Proceedings of the Ninth Meeting of the Eucarpia Section Biometrics in Plant Breeding, The Netherlands, pp. 116-124 (1994); Weber and Wricke, *Advances in Plant Breeding*, Blackwell, Berlin, 16 (1994), both of which is herein incorporated by reference in their entirety). Procedures combining interval mapping with regression analysis, whereby the phenotype is regressed onto a single putative QTL at a given marker interval and at the same time onto a number of markers that serve as 'cofactors,' have been reported by Jansen and Stam, *Genetics* 136:1447-1455 (1994), the entirety of which is herein incorporated by reference and Zeng, *Genetics* 136:1457-1468 (1994) the entirety of which is herein incorporated by reference. Generally, the use of cofactors reduces the bias and sampling error of the estimated QTL positions (Utz and Melchinger, *Biometrics in Plant Breeding*, van Oijen and Jansen (eds.) Proceedings of the Ninth Meeting of the Eucarpia Section Biometrics in Plant Breeding, The Netherlands, pp.195-204 (1994), the entirety of which is herein incorporated by reference, thereby improving the precision and efficiency of QTL mapping (Zeng, *Genetics* 136:1457-1468 (1994)). These models can be extended to multi-environment experiments to analyze genotype-environment interactions (Jansen *et al.*, *Theo. Appl. Genet.* 91:33-37 (1995), the entirety of which is herein incorporated by reference).

Selection of an appropriate mapping populations is important to map construction. The choice of appropriate mapping population depends on the type of marker systems employed

(Tanksley *et al.*, *Molecular mapping plant chromosomes. Chromosome structure and function: Impact of new concepts*, Gustafson and Appels (eds.), Plenum Press, New York, pp 157-173 (1988), the entirety of which is herein incorporated by reference). Consideration must be given to the source of parents (adapted vs. exotic) used in the mapping population. Chromosome pairing and recombination rates can be severely disturbed (suppressed) in wide crosses (adapted x exotic) and generally yield greatly reduced linkage distances. Wide crosses will usually provide segregating populations with a relatively large array of polymorphisms when compared to progeny in a narrow cross (adapted x adapted).

An  $F_2$  population is the first generation of selfing after the hybrid seed is produced. Usually a single  $F_1$  plant is selfed to generate a population segregating for all the genes in Mendelian (1:2:1) fashion. Maximum genetic information is obtained from a completely classified  $F_2$  population using a codominant marker system (Mather, *Measurement of Linkage in Heredity*, Methuen and Co., (1938), the entirety of which is herein incorporated by reference). In the case of dominant markers, progeny tests (e.g.  $F_3$ ,  $BCF_2$ ) are required to identify the heterozygotes, thus making it equivalent to a completely classified  $F_2$  population. However, this procedure is often prohibitive because of the cost and time involved in progeny testing. Progeny testing of  $F_2$  individuals is often used in map construction where phenotypes do not consistently reflect genotype (e.g. disease resistance) or where trait expression is controlled by a QTL. Segregation data from progeny test populations (e.g.  $F_3$  or  $BCF_2$ ) can be used in map construction. Marker-assisted selection can then be applied to cross progeny based on marker-trait map associations ( $F_2$ ,  $F_3$ ), where linkage groups have not been completely disassociated by recombination events (i.e., maximum disequilibrium).

Recombinant inbred lines (RIL) (genetically related lines; usually  $>F_5$ , developed from continuously selfing  $F_2$  lines towards homozygosity) can be used as a mapping population. Information obtained from dominant markers can be maximized by using RIL because all loci are homozygous or nearly so. Under conditions of tight linkage (i.e., about  $<10\%$  recombination), dominant and co-dominant markers evaluated in RIL populations provide more information per individual than either marker type in backcross populations (Reiter *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 89:1477-1481 (1992), the entirety of which is herein incorporated by reference). However, as the distance between markers becomes larger (i.e., loci become more independent), the information in RIL populations decreases dramatically when compared to codominant markers.

Backcross populations (e.g., generated from a cross between a successful variety (recurrent parent) and another variety (donor parent) carrying a trait not present in the former) can be utilized as a mapping population. A series of backcrosses to the recurrent parent can be made to recover most of its desirable traits. Thus a population is created consisting of individuals nearly like the recurrent parent but each individual carries varying amounts or mosaic of genomic regions from the donor parent. Backcross populations can be useful for mapping dominant markers if all loci in the recurrent parent are homozygous and the donor and recurrent parent have contrasting polymorphic marker alleles (Reiter *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 89:1477-1481 (1992)). Information obtained from backcross populations using either codominant or dominant markers is less than that obtained from  $F_2$  populations because one, rather than two, recombinant gametes are sampled per plant. Backcross populations, however, are more informative (at low marker saturation) when compared to RILs as the distance between linked loci increases in RIL populations (i.e. about  $15\%$  recombination). Increased

recombination can be beneficial for resolution of tight linkages, but may be undesirable in the construction of maps with low marker saturation.

Near-isogenic lines (NIL) created by many backcrosses to produce an array of individuals that are nearly identical in genetic composition except for the trait or genomic region under interrogation can be used as a mapping population. In mapping with NILs, only a portion of the polymorphic loci are expected to map to a selected region.

Bulk segregant analysis (BSA) is a method developed for the rapid identification of linkage between markers and traits of interest (Michelmore *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 88:9828-9832 (1991), the entirety of which is herein incorporated by reference). In BSA, two bulked DNA samples are drawn from a segregating population originating from a single cross. These bulks contain individuals that are identical for a particular trait (resistant or susceptible to particular disease) or genomic region but arbitrary at unlinked regions (i.e. heterozygous). Regions unlinked to the target region will not differ between the bulked samples of many individuals in BSA.

It is understood that one or more of the nucleic acid molecules of the present invention may be used as molecular markers. It is also understood that one or more of the protein molecules of the present invention may be used as molecular markers.

In accordance with this aspect of the present invention, a sample nucleic acid is obtained from plants cells or tissues. Any source of nucleic acid may be used. Preferably, the nucleic acid is genomic DNA. The nucleic acid is subjected to restriction endonuclease digestion. For example, one or more nucleic acid molecule or fragment thereof of the present invention can be used as a probe in accordance with the above-described polymorphic methods. The polymorphism obtained in this approach can then be cloned to identify the mutation at the coding

region which alters the protein's structure or regulatory region of the gene which affects its expression level.

In an aspect of the present invention, one or more of the nucleic molecules of the present invention are used to determine the level (i.e., the concentration of mRNA in a sample, etc.) in a plant (preferably maize or soybean) or pattern (i.e., the kinetics of expression, rate of decomposition, stability profile, etc.) of the expression of a protein encoded in part or whole by one or more of the nucleic acid molecule of the present invention (collectively, the "Expression Response" of a cell or tissue). As used herein, the Expression Response manifested by a cell or tissue is said to be "altered" if it differs from the Expression Response of cells or tissues of plants not exhibiting the phenotype. To determine whether a Expression Response is altered, the Expression Response manifested by the cell or tissue of the plant exhibiting the phenotype is compared with that of a similar cell or tissue sample of a plant not exhibiting the phenotype. As will be appreciated, it is not necessary to re-determine the Expression Response of the cell or tissue sample of plants not exhibiting the phenotype each time such a comparison is made; rather, the Expression Response of a particular plant may be compared with previously obtained values of normal plants. As used herein, the phenotype of the organism is any of one or more characteristics of an organism (e.g. disease resistance, pest tolerance, environmental tolerance such as tolerance to abiotic stress, male sterility, quality improvement or yield etc.). A change in genotype or phenotype may be transient or permanent. Also as used herein, a tissue sample is any sample that comprises more than one cell. In a preferred aspect, a tissue sample comprises cells that share a common characteristic (e.g. derived from root, seed, flower, leaf, stem or pollen etc.).

In one aspect of the present invention, an evaluation can be conducted to determine whether a particular mRNA molecule is present. One or more of the nucleic acid molecules of the present invention, preferably one or more of the EST nucleic acid molecules or fragments thereof of the present invention are utilized to detect the presence or quantity of the mRNA species. Such molecules are then incubated with cell or tissue extracts of a plant under conditions sufficient to permit nucleic acid hybridization. The detection of double-stranded probe-mRNA hybrid molecules is indicative of the presence of the mRNA; the amount of such hybrid formed is proportional to the amount of mRNA. Thus, such probes may be used to ascertain the level and extent of the mRNA production in a plant's cells or tissues. Such nucleic acid hybridization may be conducted under quantitative conditions (thereby providing a numerical value of the amount of the mRNA present). Alternatively, the assay may be conducted as a qualitative assay that indicates either that the mRNA is present, or that its level exceeds a user set, predefined value.

A principle of *in situ* hybridization is that a labeled, single-stranded nucleic acid probe will hybridize to a complementary strand of cellular DNA or RNA and, under the appropriate conditions, these molecules will form a stable hybrid. When nucleic acid hybridization is combined with histological techniques, specific DNA or RNA sequences can be identified within a single cell. An advantage of *in situ* hybridization over more conventional techniques for the detection of nucleic acids is that it allows an investigator to determine the precise spatial population (Angerer *et al.*, *Dev. Biol.* 101:477-484 (1984), the entirety of which is herein incorporated by reference; Angerer *et al.*, *Dev. Biol.* 112:157-166 (1985), the entirety of which is herein incorporated by reference; Dixon *et al.*, *EMBO J.* 10:1317-1324 (1991), the entirety of which is herein incorporated by reference). *In situ* hybridization may be used to measure the

steady-state level of RNA accumulation. It is a sensitive technique and RNA sequences present in as few as 5-10 copies per cell can be detected (Hardin *et al.*, *J. Mol. Biol.* 202:417-431 (1989), the entirety of which is herein incorporated by reference). A number of protocols have been devised for *in situ* hybridization, each with tissue preparation, hybridization and washing conditions (Meyerowitz, *Plant Mol. Biol. Rep.* 5:242-250 (1987), the entirety of which is herein incorporated by reference; Cox and Goldberg, In: *Plant Molecular Biology: A Practical Approach*, Shaw (ed.), pp 1-35, IRL Press, Oxford (1988), the entirety of which is herein incorporated by reference; Raikhel *et al.*, *In situ RNA hybridization in plant tissues*, In: *Plant Molecular Biology Manual*, vol. B9:1-32, Kluwer Academic Publisher, Dordrecht, Belgium (1989), the entirety of which is herein incorporated by reference).

*In situ* hybridization also allows for the localization of proteins within a tissue or cell (Wilkinson, *In Situ Hybridization*, Oxford University Press, Oxford (1992), the entirety of which is herein incorporated by reference; Langdale, *In Situ Hybridization* In: *The Maize Handbook*, Freeling and Walbot (eds.), pp 165-179, Springer-Verlag, New York (1994), the entirety of which is herein incorporated by reference). It is understood that one or more of the molecules of the present invention, preferably one or more of the EST nucleic acid molecules or fragments thereof of the present invention or one or more of the antibodies of the present invention may be utilized to detect the level or pattern of a methionine pathway protein or mRNA thereof by *in situ* hybridization.

Fluorescent *in situ* hybridization allows the localization of a particular DNA sequence along a chromosome which is useful, among other uses, for gene mapping, following chromosomes in hybrid lines or detecting chromosomes with translocations, transversions or deletions. *In situ* hybridization has been used to identify chromosomes in several plant species



(Griffor *et al.*, *Plant Mol. Biol.* 17:101-109 (1991), the entirety of which is herein incorporated by reference; Gustafson *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 87:1899-1902 (1990), herein incorporated by reference; Mukai and Gill, *Genome* 34:448-452 (1991), the entirety of which is herein incorporated by reference; Schwarzacher and Heslop-Harrison, *Genome* 34:317-323 (1991); Wang *et al.*, *Jpn. J. Genet.* 66:313-316 (1991), the entirety of which is herein incorporated by reference; Parra and Windle, *Nature Genetics* 5:17-21 (1993), the entirety of which is herein incorporated by reference). It is understood that the nucleic acid molecules of the present invention may be used as probes or markers to localize sequences along a chromosome.

Another method to localize the expression of a molecule is tissue printing. Tissue printing provides a way to screen, at the same time on the same membrane many tissue sections from different plants or different developmental stages. Tissue-printing procedures utilize films designed to immobilize proteins and nucleic acids. In essence, a freshly cut section of a tissue is pressed gently onto nitrocellulose paper, nylon membrane or polyvinylidene difluoride membrane. Such membranes are commercially available (*e.g.* Millipore, Bedford, Massachusetts U.S.A.). The contents of the cut cell transfer onto the membrane and the contents and are immobilized to the membrane. The immobilized contents form a latent print that can be visualized with appropriate probes. When a plant tissue print is made on nitrocellulose paper, the cell walls leave a physical print that makes the anatomy visible without further treatment (Varner and Taylor, *Plant Physiol.* 91:31-33 (1989), the entirety of which is herein incorporated by reference).

Tissue printing on substrate films is described by Daoust, *Exp. Cell Res.* 12:203-211 (1957), the entirety of which is herein incorporated by reference, who detected amylase, protease, ribonuclease and deoxyribonuclease in animal tissues using starch, gelatin and agar films. These

techniques can be applied to plant tissues (Yomo and Taylor, *Planta* 112:35-43 (1973); the entirety of which is herein incorporated by reference; Harris and Chrispeels, *Plant Physiol.* 56:292-299 (1975), the entirety of which is herein incorporated by reference). Advances in membrane technology have increased the range of applications of Daoust's tissue-printing techniques allowing (Cassab and Varner, *J. Cell. Biol.* 105:2581-2588 (1987), the entirety of which is herein incorporated by reference) the histochemical localization of various plant enzymes and deoxyribonuclease on nitrocellulose paper and nylon (Spruce *et al.*, *Phytochemistry* 26:2901-2903 (1987), the entirety of which is herein incorporated by reference; Barres *et al.*, *Neuron* 5:527-544 (1990), the entirety of which is herein incorporated by reference; Reid and Pont-Lezica, *Tissue Printing: Tools for the Study of Anatomy, Histochemistry and Gene Expression*, Academic Press, New York, New York (1992), the entirety of which is herein incorporated by reference; Reid *et al.*, *Plant Physiol.* 93:160-165 (1990), the entirety of which is herein incorporated by reference; Ye *et al.*, *Plant J.* 1:175-183 (1991), the entirety of which is herein incorporated by reference).

It is understood that one or more of the molecules of the present invention, preferably one or more of the EST nucleic acid molecules or fragments thereof of the present invention or one or more of the antibodies of the present invention may be utilized to detect the presence or quantity of a methionine pathway protein by tissue printing.

Further it is also understood that any of the nucleic acid molecules of the present invention may be used as marker nucleic acids and or probes in connection with methods that require probes or marker nucleic acids. As used herein, a probe is an agent that is utilized to determine an attribute or feature (e.g. presence or absence, location, correlation, etc.) of a molecule, cell, tissue or plant. As used herein, a marker nucleic acid is a nucleic acid molecule

that is utilized to determine an attribute or feature (*e.g.*, presence or absence, location, correlation, etc.) or a molecule, cell, tissue or plant.

A microarray-based method for high-throughput monitoring of plant gene expression may be utilized to measure gene-specific hybridization targets. This 'chip'-based approach involves using microarrays of nucleic acid molecules as gene-specific hybridization targets to quantitatively measure expression of the corresponding plant genes (Schena *et al.*, *Science* 270:467-470 (1995), the entirety of which is herein incorporated by reference; Shalon, Ph.D. Thesis, Stanford University (1996), the entirety of which is herein incorporated by reference). Every nucleotide in a large sequence can be queried at the same time. Hybridization can be used to efficiently analyze nucleotide sequences.

Several microarray methods have been described. One method compares the sequences to be analyzed by hybridization to a set of oligonucleotides representing all possible subsequences (Bains and Smith, *J. Theor. Biol.* 135:303-307 (1989), the entirety of which is herein incorporated by reference). A second method hybridizes the sample to an array of oligonucleotide or cDNA molecules. An array consisting of oligonucleotides complementary to subsequences of a target sequence can be used to determine the identity of a target sequence, measure its amount and detect differences between the target and a reference sequence. Nucleic acid molecules microarrays may also be screened with protein molecules or fragments thereof to determine nucleic acid molecules that specifically bind protein molecules or fragments thereof.

The microarray approach may be used with polypeptide targets (U.S. Patent No. 5,445,934; U.S. Patent No: 5,143,854; U.S. Patent No. 5,079,600; U.S. Patent No. 4,923,901, all of which are herein incorporated by reference in their entirety). Essentially, polypeptides are synthesized on a substrate (microarray) and these polypeptides can be screened with either

protein molecules or fragments thereof or nucleic acid molecules in order to screen for either protein molecules or fragments thereof or nucleic acid molecules that specifically bind the target polypeptides. (Fodor *et al.*, *Science* 251:767-773 (1991), the entirety of which is herein incorporated by reference). It is understood that one or more of the nucleic acid molecules or protein or fragments thereof of the present invention may be utilized in a microarray based method.

In a preferred embodiment of the present invention microarrays may be prepared that comprise nucleic acid molecules where such nucleic acid molecules encode at least one, preferably at least two, more preferably at least three, even more preferably at least four, five six or seven methionine pathway enzymes. In a preferred embodiment the nucleic acid molecules are selected from the group consisting of a nucleic acid molecule that encodes a maize or a soybean methionine adenosyltransferase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean S-adenosylmethionine decarboxylase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean aspartate kinase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean aspartate-semialdehyde dehydrogenase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean *O*-succinylhomoserine (thiol)-lyase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean cystathionine  $\beta$ -lyase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean 5-methyltetrahydropteroyltriglutamate-homocysteine-S-methyltransferase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean adenosylhomocysteinase enzyme or fragment thereof, a nucleic acid molecule that encodes a maize or a soybean cystathionine  $\beta$ -synthase enzyme or fragment thereof, a nucleic acid molecule that encodes a

maize or a soybean cystathionine  $\gamma$ -lyase enzyme or fragment thereof and a nucleic acid molecule that encodes a maize or a soybean *O*-acetylhomoserine (thiol)-lyase enzyme or fragment thereof.

Site directed mutagenesis may be utilized to modify nucleic acid sequences, particularly as it is a technique that allows one or more of the amino acids encoded by a nucleic acid molecule to be altered (e.g. a threonine to be replaced by a methionine). Three basic methods for site directed mutagenesis are often employed. These are cassette mutagenesis (Wells *et al.*, *Gene* 34:315-323 (1985), the entirety of which is herein incorporated by reference), primer extension (Gilliam *et al.*, *Gene* 12:129-137 (1980), the entirety of which is herein incorporated by reference; Zoller and Smith, *Methods Enzymol.* 100:468-500 (1983), the entirety of which is herein incorporated by reference; Dalbadie-McFarland *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 79:6409-6413 (1982), the entirety of which is herein incorporated by reference) and methods based upon PCR (Scharf *et al.*, *Science* 233:1076-1078 (1986), the entirety of which is herein incorporated by reference; Higuchi *et al.*, *Nucleic Acids Res.* 16:7351-7367 (1988), the entirety of which is herein incorporated by reference). Site directed mutagenesis approaches are also described in European Patent 0 385 962, the entirety of which is herein incorporated by reference; European Patent 0 359 472, the entirety of which is herein incorporated by reference; and PCT Patent Application WO 93/07278, the entirety of which is herein incorporated by reference.

Site directed mutagenesis strategies have been applied to plants for both *in vitro* as well as *in vivo* site directed mutagenesis (Lanz *et al.*, *J. Biol. Chem.* 266:9971-9976 (1991), the entirety of which is herein incorporated by reference; Kovgan and Zhdanov, *Biotekhnologiya* 5:148-154, No. 207160n, Chemical Abstracts 110:225 (1989), the entirety of which is herein incorporated by reference; Ge *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 86:4037-4041 (1989), the

entirety of which is herein incorporated by reference; Zhu *et al.*, *J. Biol. Chem.* 271:18494-18498 (1996), the entirety of which is herein incorporated by reference; Chu *et al.*, *Biochemistry* 33:6150-6157 (1994), the entirety of which is herein incorporated by reference; Small *et al.*, *EMBO J.* 11:1291-1296 (1992), the entirety of which is herein incorporated by reference; Cho *et al.*, *Mol. Biotechnol.* 8:13-16 (1997), the entirety of which is herein incorporated by reference; Kita *et al.*, *J. Biol. Chem.* 271:26529-26535 (1996), the entirety of which is herein incorporated by reference; Jin *et al.*, *Mol. Microbiol.* 7:555-562 (1993), the entirety of which is herein incorporated by reference; Hatfield and Vierstra, *J. Biol. Chem.* 267:14799-14803 (1992), the entirety of which is herein incorporated by reference; Zhao *et al.*, *Biochemistry* 31:5093-5099 (1992), the entirety of which is herein incorporated by reference).

Any of the nucleic acid molecules of the present invention may either be modified by site directed mutagenesis or used as, for example, nucleic acid molecules that are used to target other nucleic acid molecules for modification. It is understood that mutants with more than one altered nucleotide can be constructed using techniques that practitioners are familiar with such as isolating restriction fragments and ligating such fragments into an expression vector (*see, for example, Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1989).*).

Sequence-specific DNA-binding proteins play a role in the regulation of transcription. The isolation of recombinant cDNAs encoding these proteins facilitates the biochemical analysis of their structural and functional properties. Genes encoding such DNA-binding proteins have been isolated using classical genetics (Vollbrecht *et al.*, *Nature* 350: 241-243 (1991), the entirety of which is herein incorporated by reference) and molecular biochemical approaches, including the screening of recombinant cDNA libraries with antibodies (Landschulz *et al.*, *Genes Dev.*

2:786-800 (1988), the entirety of which is herein incorporated by reference) or DNA probes (Bodner *et al.*, *Cell* 55:505-518 (1988), the entirety of which is herein incorporated by reference). In addition, an *in situ* screening procedure has been used and has facilitated the isolation of sequence-specific DNA-binding proteins from various plant species (Gilmartin *et al.*, *Plant Cell* 4:839-849 (1992), the entirety of which is herein incorporated by reference; Schindler *et al.*, *EMBO J.* 11:1261-1273 (1992), the entirety of which is herein incorporated by reference). An *in situ* screening protocol does not require the purification of the protein of interest (Vinson *et al.*, *Genes Dev.* 2:801-806 (1988), the entirety of which is herein incorporated by reference; Singh *et al.*, *Cell* 52:415-423 (1988), the entirety of which is herein incorporated by reference).

Two steps may be employed to characterize DNA-protein interactions. The first is to identify promoter fragments that interact with DNA-binding proteins, to titrate binding activity, to determine the specificity of binding and to determine whether a given DNA-binding activity can interact with related DNA sequences (Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2<sup>nd</sup> edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989)). Electrophoretic mobility-shift assay is a widely used assay. The assay provides a rapid and sensitive method for detecting DNA-binding proteins based on the observation that the mobility of a DNA fragment through a nondenaturing, low-ionic strength polyacrylamide gel is retarded upon association with a DNA-binding protein (Fried and Crother, *Nucleic Acids Res.* 9:6505-6525 (1981), the entirety of which is herein incorporated by reference). When one or more specific binding activities have been identified, the exact sequence of the DNA bound by the protein may be determined. Several procedures for characterizing protein/DNA-binding sites are used, including methylation and ethylation interference assays (Maxam and Gilbert, *Methods*

*Enzymol.* 65:499-560 (1980), the entirety of which is herein incorporated by reference; Wissman and Hillen, *Methods Enzymol.* 208:365-379 (1991), the entirety of which is herein incorporated by reference), footprinting techniques employing DNase I (Galas and Schmitz, *Nucleic Acids Res.* 5:3157-3170 (1978), the entirety of which is herein incorporated by reference), 1,10-phenanthroline-copper ion methods (Sigman *et al.*, *Methods Enzymol.* 208:414-433 (1991), the entirety of which is herein incorporated by reference) and hydroxyl radicals methods (Dixon *et al.*, *Methods Enzymol.* 208:414-433 (1991), the entirety of which is herein incorporated by reference). It is understood that one or more of the nucleic acid molecules of the present invention may be utilized to identify a protein or fragment thereof that specifically binds to a nucleic acid molecule of the present invention. It is also understood that one or more of the protein molecules or fragments thereof of the present invention may be utilized to identify a nucleic acid molecule that specifically binds to it.

A two-hybrid system is based on the fact that many cellular functions are carried out by proteins, such as transcription factors, that interact (physically) with one another. Two-hybrid systems have been used to probe the function of new proteins (Chien *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 88:9578-9582 (1991) the entirety of which is herein incorporated by reference; Durfee *et al.*, *Genes Dev.* 7:555-569 (1993) the entirety of which is herein incorporated by reference; Choi *et al.*, *Cell* 78:499-512 (1994), the entirety of which is herein incorporated by reference; Kranz *et al.*, *Genes Dev.* 8:313-327 (1994), the entirety of which is herein incorporated by reference).

Interaction mating techniques have facilitated a number of two-hybrid studies of protein-protein interaction. Interaction mating has been used to examine interactions between small sets of tens of proteins (Finley and Brent, *Proc. Natl. Acad. Sci. (U.S.A.)* 91:12098-12984 (1994), the entirety of which is herein incorporated by reference), larger sets of hundreds of proteins



(Bendixen *et al.*, *Nucl. Acids Res.* 22:1778-1779 (1994), the entirety of which is herein incorporated by reference) and to comprehensively map proteins encoded by a small genome (Bartel *et al.*, *Nature Genetics* 12:72-77 (1996), the entirety of which is herein incorporated by reference). This technique utilizes proteins fused to the DNA-binding domain and proteins fused to the activation domain. They are expressed in two different haploid yeast strains of opposite mating type and the strains are mated to determine if the two proteins interact. Mating occurs when haploid yeast strains come into contact and result in the fusion of the two haploids into a diploid yeast strain. An interaction can be determined by the activation of a two-hybrid reporter gene in the diploid strain. An advantage of this technique is that it reduces the number of yeast transformations needed to test individual interactions. It is understood that the protein-protein interactions of protein or fragments thereof of the present invention may be investigated using the two-hybrid system and that any of the nucleic acid molecules of the present invention that encode such proteins or fragments thereof may be used to transform yeast in the two-hybrid system.

#### **(a) Plant Constructs and Plant Transformants**

One or more of the nucleic acid molecules of the present invention may be used in plant transformation or transfection. Exogenous genetic material may be transferred into a plant cell and the plant cell regenerated into a whole, fertile or sterile plant. Exogenous genetic material is any genetic material, whether naturally occurring or otherwise, from any source that is capable of being inserted into any organism. Such genetic material may be transferred into either monocotyledons and dicotyledons including, but not limited to maize (pp 63-69), soybean (pp

50-60), *Arabidopsis* (p 45), phaseolus (pp 47-49), peanut (pp 49-50), alfalfa (p 60), wheat (pp 69-71), rice (pp 72-79), oat (pp 80-81), sorghum (p 83), rye (p 84), tritordeum (p 84), millet (p85), fescue (p 85), perennial ryegrass (p 86), sugarcane (p87), cranberry (p101), papaya (pp 101-102), banana (p 103), banana (p 103), muskmelon (p 104), apple (p 104), cucumber (p 105), dendrobium (p 109), gladiolus (p 110), chrysanthemum (p 110), liliacea (p 111), cotton (pp113-114), eucalyptus (p 115), sunflower (p 118), canola (p 118), turfgrass (p121), sugarbeet (p 122), coffee (p 122) and dioscorea (p 122), (Christou, In: *Particle Bombardment for Genetic Engineering of Plants*, Biotechnology Intelligence Unit. Academic Press, San Diego, California (1996), the entirety of which is herein incorporated by reference).

Transfer of a nucleic acid that encodes for a protein can result in overexpression of that protein in a transformed cell or transgenic plant. One or more of the proteins or fragments thereof encoded by nucleic acid molecules of the present invention may be overexpressed in a transformed cell or transformed plant. Particularly, any of the methionine pathway proteins or fragments thereof may be overexpressed in a transformed cell or transgenic plant. Such overexpression may be the result of transient or stable transfer of the exogenous genetic material.

Exogenous genetic material may be transferred into a plant cell and the plant cell by the use of a DNA vector or construct designed for such a purpose. Design of such a vector is generally within the skill of the art (See, *Plant Molecular Biology: A Laboratory Manual*, Clark (ed.), Springer, New York (1997), the entirety of which is herein incorporated by reference).

A construct or vector may include a plant promoter to express the protein or protein fragment of choice. A number of promoters which are active in plant cells have been described in the literature. These include the nopaline synthase (NOS) promoter (Ebert *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 84:5745-5749 (1987), the entirety of which is herein incorporated by

reference), the octopine synthase (OCS) promoter (which are carried on tumor-inducing plasmids of *Agrobacterium tumefaciens*), the caulimovirus promoters such as the cauliflower mosaic virus (CaMV) 19S promoter (Lawton *et al.*, *Plant Mol. Biol.* 9:315-324 (1987), the entirety of which is herein incorporated by reference) and the CAMV 35S promoter (Odell *et al.*, *Nature* 313:810-812 (1985), the entirety of which is herein incorporated by reference), the figwort mosaic virus 35S-promoter, the light-inducible promoter from the small subunit of ribulose-1,5-bis-phosphate carboxylase (ssRUBISCO), the Adh promoter (Walker *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 84:6624-6628 (1987), the entirety of which is herein incorporated by reference), the sucrose synthase promoter (Yang *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 87:4144-4148 (1990), the entirety of which is herein incorporated by reference), the R gene complex promoter (Chandler *et al.*, *The Plant Cell* 1:1175-1183 (1989), the entirety of which is herein incorporated by reference) and the chlorophyll a/b binding protein gene promoter, etc. These promoters have been used to create DNA constructs which have been expressed in plants; *see, e.g.*, PCT publication WO 84/02913, herein incorporated by reference in its entirety.

Promoters which are known or are found to cause transcription of DNA in plant cells can be used in the present invention. Such promoters may be obtained from a variety of sources such as plants and plant viruses. It is preferred that the particular promoter selected should be capable of causing sufficient expression to result in the production of an effective amount of the methionine pathway protein to cause the desired phenotype. In addition to promoters that are known to cause transcription of DNA in plant cells, other promoters may be identified for use in the current invention by screening a plant cDNA library for genes which are selectively or preferably expressed in the target tissues or cells.

For the purpose of expression in source tissues of the plant, such as the leaf, seed, root or stem, it is preferred that the promoters utilized in the present invention have relatively high expression in these specific tissues. For this purpose, one may choose from a number of promoters for genes with tissue- or cell-specific or -enhanced expression. Examples of such promoters reported in the literature include the chloroplast glutamine synthetase GS2 promoter from pea (Edwards *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 87:3459-3463 (1990), herein incorporated by reference in its entirety), the chloroplast fructose-1,6-biphosphatase (FBPase) promoter from wheat (Lloyd *et al.*, *Mol. Gen. Genet.* 225:209-216 (1991), herein incorporated by reference in its entirety), the nuclear photosynthetic ST-LS1 promoter from potato (Stockhaus *et al.*, *EMBO J.* 8:2445-2451 (1989), herein incorporated by reference in its entirety), the serine/threonine kinase (PAL) promoter and the glucoamylase (CHS) promoter from *Arabidopsis thaliana*. Also reported to be active in photosynthetically active tissues are the ribulose-1,5-bisphosphate carboxylase (RbcS) promoter from eastern larch (*Larix laricina*), the promoter for the *cab* gene, *cab6*, from pine (Yamamoto *et al.*, *Plant Cell Physiol.* 35:773-778 (1994), herein incorporated by reference in its entirety), the promoter for the *Cab-1* gene from wheat (Fejes *et al.*, *Plant Mol. Biol.* 15:921-932 (1990), herein incorporated by reference in its entirety), the promoter for the *CAB-1* gene from spinach (Lubberstedt *et al.*, *Plant Physiol.* 104:997-1006 (1994), herein incorporated by reference in its entirety), the promoter for the *cab1R* gene from rice (Luan *et al.*, *Plant Cell.* 4:971-981 (1992), the entirety of which is herein incorporated by reference), the pyruvate, orthophosphate dikinase (PPDK) promoter from maize (Matsuoka *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 90: 9586-9590 (1993), herein incorporated by reference in its entirety), the promoter for the tobacco *Lhcb1\*2* gene (Cerdan *et al.*, *Plant Mol. Biol.* 33:245-255 (1997), herein incorporated by reference in its entirety), the *Arabidopsis thaliana* SUC2 sucrose-

H<sup>+</sup> symporter promoter (Truernit *et al.*, *Planta*. 196:564-570 (1995), herein incorporated by reference in its entirety) and the promoter for the thylakoid membrane proteins from spinach (psaD, psaF, psaE, PC, FNR, atpC, atpD, cab, rbcS). Other promoters for the chlorophyll a/b-binding proteins may also be utilized in the present invention, such as the promoters for LhcB gene and PsbP gene from white mustard (*Sinapis alba*; Kretsch *et al.*, *Plant Mol. Biol.* 28:219-229 (1995), the entirety of which is herein incorporated by reference).

For the purpose of expression in sink tissues of the plant, such as the tuber of the potato plant, the fruit of tomato, or the seed of maize, wheat, rice and barley, it is preferred that the promoters utilized in the present invention have relatively high expression in these specific tissues. A number of promoters for genes with tuber-specific or -enhanced expression are known, including the class I patatin promoter (Bevan *et al.*, *EMBO J.* 8:1899-1906 (1986); Jefferson *et al.*, *Plant Mol. Biol.* 14:995-1006 (1990), both of which are herein incorporated by reference in its entirety), the promoter for the potato tuber ADPGPP genes, both the large and small subunits, the sucrose synthase promoter (Salanoubat and Belliard, *Gene*. 60:47-56 (1987), Salanoubat and Belliard, *Gene*. 84:181-185 (1989), both of which are incorporated by reference in their entirety), the promoter for the major tuber proteins including the 22 kd protein complexes and proteinase inhibitors (Hannapel, *Plant Physiol.* 101:703-704 (1993), herein incorporated by reference in its entirety), the promoter for the granule bound starch synthase gene (GBSS) (Visser *et al.*, *Plant Mol. Biol.* 17:691-699 (1991), herein incorporated by reference in its entirety) and other class I and II patatins promoters (Koster-Topfer *et al.*, *Mol Gen Genet.* 219:390-396 (1989); Mignery *et al.*, *Gene*. 62:27-44 (1988), both of which are herein incorporated by reference in their entirety).

Other promoters can also be used to express a methionine pathway protein or fragment thereof in specific tissues, such as seeds or fruits. The promoter for  $\beta$ -conglycinin (Chen *et al.*, *Dev. Genet.* 10: 112-122 (1989), herein incorporated by reference in its entirety) or other seed-specific promoters such as the napin and phaseolin promoters, can be used. The zeins are a group of storage proteins found in maize endosperm. Genomic clones for zein genes have been isolated (Pedersen *et al.*, *Cell* 29:1015-1026 (1982), herein incorporated by reference in its entirety) and the promoters from these clones, including the 15 kD, 16 kD, 19 kD, 22 kD, 27 kD and  $\gamma$  genes, could also be used. Other promoters known to function, for example, in maize include the promoters for the following genes: *waxy*, *Brittle*, *Shrunken 2*, Branching enzymes I and II, starch synthases, debranching enzymes, oleosins, glutelins and sucrose synthases. A particularly preferred promoter for maize endosperm expression is the promoter for the glutelin gene from rice, more particularly the Osgt-1 promoter (Zheng *et al.*, *Mol. Cell Biol.* 13:5829-5842 (1993), herein incorporated by reference in its entirety). Examples of promoters suitable for expression in wheat include those promoters for the ADPGlucose pyrosynthase (ADPGPP) subunits, the granule bound and other starch synthase, the branching and debranching enzymes, the embryogenesis-abundant proteins, the gliadins and the glutenins. Examples of such promoters in rice include those promoters for the ADPGPP subunits, the granule bound and other starch synthase, the branching enzymes, the debranching enzymes, sucrose synthases and the glutelins. A particularly preferred promoter is the promoter for rice glutelin, Osgt-1. Examples of such promoters for barley include those for the ADPGPP subunits, the granule bound and other starch synthase, the branching enzymes, the debranching enzymes, sucrose synthases, the hordeins, the embryo globulins and the aleurone specific proteins.

Root specific promoters may also be used. An example of such a promoter is the promoter for the acid chitinase gene (Samac *et al.*, *Plant Mol. Biol.* 25:587-596 (1994), the entirety of which is herein incorporated by reference). Expression in root tissue could also be accomplished by utilizing the root specific subdomains of the CaMV35S promoter that have been identified (Lam *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 86:7890-7894 (1989), herein incorporated by reference in its entirety). Other root cell specific promoters include those reported by Conkling *et al.* (Conkling *et al.*, *Plant Physiol.* 93:1203-1211 (1990), the entirety of which is herein incorporated by reference).

Additional promoters that may be utilized are described, for example, in U.S. Patent Nos. 5,378,619; 5,391,725; 5,428,147; 5,447,858; 5,608,144; 5,608,144; 5,614,399; 5,633,441; 5,633,435; and 4,633,436, all of which are herein incorporated in their entirety. In addition, a tissue specific enhancer may be used (Fromm *et al.*, *The Plant Cell* 1:977-984 (1989), the entirety of which is herein incorporated by reference).

Constructs or vectors may also include with the coding region of interest a nucleic acid sequence that acts, in whole or in part, to terminate transcription of that region. For example, such sequences have been isolated including the Tr7 3' sequence and the NOS 3' sequence (Ingelbrecht *et al.*, *The Plant Cell* 1:671-680 (1989), the entirety of which is herein incorporated by reference; Bevan *et al.*, *Nucleic Acids Res.* 11:369-385 (1983), the entirety of which is herein incorporated by reference), or the like.

A vector or construct may also include regulatory elements. Examples of such include the Adh intron 1 (Callis *et al.*, *Genes and Develop.* 1:1183-1200 (1987), the entirety of which is herein incorporated by reference), the sucrose synthase intron (Vasil *et al.*, *Plant Physiol.* 91:1575-1579 (1989), the entirety of which is herein incorporated by reference) and the TMV

omega element (Gallie *et al.*, *The Plant Cell* 1:301-311 (1989), the entirety of which is herein incorporated by reference). These and other regulatory elements may be included when appropriate.

A vector or construct may also include a selectable marker. Selectable markers may also be used to select for plants or plant cells that contain the exogenous genetic material. Examples of such include, but are not limited to, a neo gene (Potrykus *et al.*, *Mol. Gen. Genet.* 199:183-188 (1985), the entirety of which is herein incorporated by reference) which codes for kanamycin resistance and can be selected for using kanamycin, G418, etc.; a bar gene which codes for bialaphos resistance; a mutant EPSP synthase gene (Hinchee *et al.*, *Bio/Technology* 6:915-922 (1988), the entirety of which is herein incorporated by reference) which encodes glyphosate resistance; a nitrilase gene which confers resistance to bromoxynil (Stalker *et al.*, *J. Biol. Chem.* 263:6310-6314 (1988), the entirety of which is herein incorporated by reference); a mutant acetolactate synthase gene (ALS) which confers imidazolinone or sulphonylurea resistance (European Patent Application 154,204 (Sept. 11, 1985), the entirety of which is herein incorporated by reference); and a methotrexate resistant DHFR gene (Thillet *et al.*, *J. Biol. Chem.* 263:12500-12508 (1988), the entirety of which is herein incorporated by reference).

A vector or construct may also include a transit peptide. Incorporation of a suitable chloroplast transit peptide may also be employed (European Patent Application Publication Number 0218571, the entirety of which is herein incorporated by reference). Translational enhancers may also be incorporated as part of the vector DNA. DNA constructs could contain one or more 5' non-translated leader sequences which may serve to enhance expression of the gene products from the resulting mRNA transcripts. Such sequences may be derived from the promoter selected to express the gene or can be specifically modified to increase translation of



the mRNA. Such regions may also be obtained from viral RNAs, from suitable eukaryotic genes, or from a synthetic gene sequence. For a review of optimizing expression of transgenes, see Koziel *et al.*, *Plant Mol. Biol.* 32:393-405 (1996), the entirety of which is herein incorporated by reference.

A vector or construct may also include a screenable marker. Screenable markers may be used to monitor expression. Exemplary screenable markers include a  $\beta$ -glucuronidase or uidA gene (GUS) which encodes an enzyme for which various chromogenic substrates are known (Jefferson, *Plant Mol. Biol. Rep.* 5:387-405 (1987), the entirety of which is herein incorporated by reference; Jefferson *et al.*, *EMBO J.* 6:3901-3907 (1987), the entirety of which is herein incorporated by reference); an R-locus gene, which encodes a product that regulates the production of anthocyanin pigments (red color) in plant tissues (Dellaporta *et al.*, *Stadler Symposium* 11:263-282 (1988), the entirety of which is herein incorporated by reference); a  $\beta$ -lactamase gene (Sutcliffe *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 75:3737-3741 (1978), the entirety of which is herein incorporated by reference), a gene which encodes an enzyme for which various chromogenic substrates are known (e.g., PADAC, a chromogenic cephalosporin); a luciferase gene (Ow *et al.*, *Science* 234:856-859 (1986), the entirety of which is herein incorporated by reference); a xylE gene (Zukowsky *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 80:1101-1105 (1983), the entirety of which is herein incorporated by reference) which encodes a catechol dioxygenase that can convert chromogenic catechols; an  $\alpha$ -amylase gene (Ikata *et al.*, *Bio/Technol.* 8:241-242 (1990), the entirety of which is herein incorporated by reference); a tyrosinase gene (Katz *et al.*, *J. Gen. Microbiol.* 129:2703-2714 (1983), the entirety of which is herein incorporated by reference) which encodes an enzyme capable of oxidizing tyrosine to

DOPA and dopaquinone which in turn condenses to melanin; an  $\alpha$ -galactosidase, which will turn a chromogenic  $\alpha$ -galactose substrate.

Included within the terms “selectable or screenable marker genes” are also genes which encode a secretable marker whose secretion can be detected as a means of identifying or selecting for transformed cells. Examples include markers which encode a secretable antigen that can be identified by antibody interaction, or even secretable enzymes which can be detected catalytically. Secretable proteins fall into a number of classes, including small, diffusible proteins which are detectable, (e.g., by ELISA), small active enzymes which are detectable in extracellular solution (e.g.,  $\alpha$ -amylase,  $\beta$ -lactamase, phosphinothricin transferase), or proteins which are inserted or trapped in the cell wall (such as proteins which include a leader sequence such as that found in the expression unit of extension or tobacco PR-S). Other possible selectable and/or screenable marker genes will be apparent to those of skill in the art.

There are many methods for introducing transforming nucleic acid molecules into plant cells. Suitable methods are believed to include virtually any method by which nucleic acid molecules may be introduced into a cell, such as by *Agrobacterium* infection or direct delivery of nucleic acid molecules such as, for example, by PEG-mediated transformation, by electroporation or by acceleration of DNA coated particles, etc (Potrykus, *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 42:205-225 (1991), the entirety of which is herein incorporated by reference; Vasil, *Plant Mol. Biol.* 25:925-937 (1994), the entirety of which is herein incorporated by reference). For example, electroporation has been used to transform maize protoplasts (Fromm *et al.*, *Nature* 312:791-793 (1986), the entirety of which is herein incorporated by reference).

Other vector systems suitable for introducing transforming DNA into a host plant cell include but are not limited to binary artificial chromosome (BIBAC) vectors (Hamilton *et al.*, *Gene* 200:107-116 (1997), the entirety of which is herein incorporated by reference); and transfection with RNA viral vectors (Della-Cioppa *et al.*, *Ann. N.Y. Acad. Sci.* (1996), 792 (Engineering Plants for Commercial Products and Applications), 57-61, the entirety of which is herein incorporated by reference). Additional vector systems also include plant selectable YAC vectors such as those described in Mullen *et al.*, *Molecular Breeding* 4:449-457 (1988), the entirety of which is herein incorporated by reference).

Technology for introduction of DNA into cells is well known to those of skill in the art. Four general methods for delivering a gene into cells have been described: (1) chemical methods (Graham and van der Eb, *Virology* 54:536-539 (1973), the entirety of which is herein incorporated by reference); (2) physical methods such as microinjection (Capecchi, *Cell* 22:479-488 (1980), the entirety of which is herein incorporated by reference), electroporation (Wong and Neumann, *Biochem. Biophys. Res. Commun.* 107:584-587 (1982); Fromm *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 82:5824-5828 (1985); U.S. Patent No. 5,384,253, all of which are herein incorporated in their entirety); and the gene gun (Johnston and Tang, *Methods Cell Biol.* 43:353-365 (1994), the entirety of which is herein incorporated by reference); (3) viral vectors (Clapp, *Clin. Perinatol.* 20:155-168 (1993); Lu *et al.*, *J. Exp. Med.* 178:2089-2096 (1993); Eglitis and Anderson, *Biotechniques* 6:608-614 (1988), all of which are herein incorporated in their entirety); and (4) receptor-mediated mechanisms (Curiel *et al.*, *Hum. Gen. Ther.* 3:147-154 (1992), Wagner *et al.*, *Proc. Natl. Acad. Sci. (USA)* 89:6099-6103 (1992), both of which are incorporated by reference in their entirety).

Acceleration methods that may be used include, for example, microprojectile bombardment and the like. One example of a method for delivering transforming nucleic acid molecules to plant cells is microprojectile bombardment. This method has been reviewed by Yang and Christou (eds.), *Particle Bombardment Technology for Gene Transfer*, Oxford Press, Oxford, England (1994), the entirety of which is herein incorporated by reference). Non-biological particles (microprojectiles) that may be coated with nucleic acids and delivered into cells by a propelling force. Exemplary particles include those comprised of tungsten, gold, platinum and the like.

A particular advantage of microprojectile bombardment, in addition to it being an effective means of reproducibly transforming monocots, is that neither the isolation of protoplasts (Cristou *et al.*, *Plant Physiol.* 87:671-674 (1988), the entirety of which is herein incorporated by reference) nor the susceptibility of *Agrobacterium* infection are required. An illustrative embodiment of a method for delivering DNA into maize cells by acceleration is a biolistics  $\alpha$ -particle delivery system, which can be used to propel particles coated with DNA through a screen, such as a stainless steel or Nytex screen, onto a filter surface covered with corn cells cultured in suspension. Gordon-Kamm *et al.*, describes the basic procedure for coating tungsten particles with DNA (Gordon-Kamm *et al.*, *Plant Cell* 2:603-618 (1990), the entirety of which is herein incorporated by reference). The screen disperses the tungsten nucleic acid particles so that they are not delivered to the recipient cells in large aggregates. A particle delivery system suitable for use with the present invention is the helium acceleration PDS-1000/He gun is available from Bio-Rad Laboratories (Bio-Rad, Hercules, California)(Sanford *et al.*, *Technique* 3:3-16 (1991), the entirety of which is herein incorporated by reference).

For the bombardment, cells in suspension may be concentrated on filters. Filters containing the cells to be bombarded are positioned at an appropriate distance below the microprojectile stopping plate. If desired, one or more screens are also positioned between the gun and the cells to be bombarded.

Alternatively, immature embryos or other target cells may be arranged on solid culture medium. The cells to be bombarded are positioned at an appropriate distance below the microprojectile stopping plate. If desired, one or more screens are also positioned between the acceleration device and the cells to be bombarded. Through the use of techniques set forth herein one may obtain up to 1000 or more foci of cells transiently expressing a marker gene. The number of cells in a focus which express the exogenous gene product 48 hours post-bombardment often range from one to ten and average one to three.

In bombardment transformation, one may optimize the pre-bombardment culturing conditions and the bombardment parameters to yield the maximum numbers of stable transformants. Both the physical and biological parameters for bombardment are important in this technology. Physical factors are those that involve manipulating the DNA/microprojectile precipitate or those that affect the flight and velocity of either the macro- or microprojectiles. Biological factors include all steps involved in manipulation of cells before and immediately after bombardment, the osmotic adjustment of target cells to help alleviate the trauma associated with bombardment and also the nature of the transforming DNA, such as linearized DNA or intact supercoiled plasmids. It is believed that pre-bombardment manipulations are especially important for successful transformation of immature embryos.

In another alternative embodiment, plastids can be stably transformed. Methods disclosed for plastid transformation in higher plants include the particle gun delivery of DNA

containing a selectable marker and targeting of the DNA to the plastid genome through homologous recombination (Svab *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 87:8526-8530 (1990); Svab and Maliga, *Proc. Natl. Acad. Sci. (U.S.A.)* 90:913-917 (1993); Staub and Maliga, *EMBO J.* 12:601-606 (1993); U.S. Patents 5,451,513 and 5,545,818, all of which are herein incorporated by reference in their entirety).

Accordingly, it is contemplated that one may wish to adjust various aspects of the bombardment parameters in small scale studies to fully optimize the conditions. One may particularly wish to adjust physical parameters such as gap distance, flight distance, tissue distance and helium pressure. One may also minimize the trauma reduction factors by modifying conditions which influence the physiological state of the recipient cells and which may therefore influence transformation and integration efficiencies. For example, the osmotic state, tissue hydration and the subculture stage or cell cycle of the recipient cells may be adjusted for optimum transformation. The execution of other routine adjustments will be known to those of skill in the art in light of the present disclosure.

*Agrobacterium*-mediated transfer is a widely applicable system for introducing genes into plant cells because the DNA can be introduced into whole plant tissues, thereby bypassing the need for regeneration of an intact plant from a protoplast. The use of *Agrobacterium*-mediated plant integrating vectors to introduce DNA into plant cells is well known in the art. See, for example the methods described by Fraley *et al.*, *Bio/Technology* 3:629-635 (1985) and Rogers *et al.*, *Methods Enzymol.* 153:253-277 (1987), both of which are herein incorporated by reference in their entirety. Further, the integration of the Ti-DNA is a relatively precise process resulting in few rearrangements. The region of DNA to be transferred is defined by the border sequences and

intervening DNA is usually inserted into the plant genome as described (Spielmann *et al.*, *Mol. Gen. Genet.* 205:34 (1986), the entirety of which is herein incorporated by reference).

Modern *Agrobacterium* transformation vectors are capable of replication in *E. coli* as well as *Agrobacterium*, allowing for convenient manipulations as described (Klee *et al.*, In: *Plant DNA Infectious Agents*, Hohn and Schell (eds.), Springer-Verlag, New York, pp. 179-203 (1985), the entirety of which is herein incorporated by reference. Moreover, technological advances in vectors for *Agrobacterium*-mediated gene transfer have improved the arrangement of genes and restriction sites in the vectors to facilitate construction of vectors capable of expressing various polypeptide coding genes. The vectors described have convenient multi-linker regions flanked by a promoter and a polyadenylation site for direct expression of inserted polypeptide coding genes and are suitable for present purposes (Rogers *et al.*, *Methods Enzymol.* 153:253-277 (1987)). In addition, *Agrobacterium* containing both armed and disarmed Ti genes can be used for the transformations. In those plant strains where *Agrobacterium*-mediated transformation is efficient, it is the method of choice because of the facile and defined nature of the gene transfer.

A transgenic plant formed using *Agrobacterium* transformation methods typically contains a single gene on one chromosome. Such transgenic plants can be referred to as being heterozygous for the added gene. More preferred is a transgenic plant that is homozygous for the added structural gene; *i.e.*, a transgenic plant that contains two added genes, one gene at the same locus on each chromosome of a chromosome pair. A homozygous transgenic plant can be obtained by sexually mating (selfing) an independent segregant transgenic plant that contains a single added gene, germinating some of the seed produced and analyzing the resulting plants produced for the gene of interest.

It is also to be understood that two different transgenic plants can also be mated to produce offspring that contain two independently segregating added, exogenous genes. Selfing of appropriate progeny can produce plants that are homozygous for both added, exogenous genes that encode a polypeptide of interest. Back-crossing to a parental plant and out-crossing with a non-transgenic plant are also contemplated, as is vegetative propagation.

Transformation of plant protoplasts can be achieved using methods based on calcium phosphate precipitation, polyethylene glycol treatment, electroporation and combinations of these treatments (*See, for example, Potrykus et al., Mol. Gen. Genet.* 205:193-200 (1986); Lorz *et al., Mol. Gen. Genet.* 199:178 (1985); Fromm *et al., Nature* 319:791 (1986); Uchimiya *et al., Mol. Gen. Genet.* 204:204 (1986); Marcotte *et al., Nature* 335:454-457 (1988), all of which are herein incorporated by reference in their entirety).

Application of these systems to different plant strains depends upon the ability to regenerate that particular plant strain from protoplasts. Illustrative methods for the regeneration of cereals from protoplasts are described (Fujimura *et al., Plant Tissue Culture Letters* 2:74 (1985); Toriyama *et al., Theor Appl. Genet.* 205:34 (1986); Yamada *et al., Plant Cell Rep.* 4:85 (1986); Abdullah *et al., Biotechnolog* 4:1087 (1986), all of which are herein incorporated by reference in their entirety).

To transform plant strains that cannot be successfully regenerated from protoplasts, other ways to introduce DNA into intact cells or tissues can be utilized. For example, regeneration of cereals from immature embryos or explants can be effected as described (Vasil, *Biotechnology* 6:397 (1988), the entirety of which is herein incorporated by reference). In addition, "particle gun" or high-velocity microprojectile technology can be utilized (Vasil *et al., Bio/Technology* 10:667 (1992), the entirety of which is herein incorporated by reference).



Using the latter technology, DNA is carried through the cell wall and into the cytoplasm on the surface of small metal particles as described (Klein *et al.*, *Nature* 328:70 (1987); Klein *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 85:8502-8505 (1988); McCabe *et al.*, *Bio/Technology* 6:923 (1988), all of which are herein incorporated by reference in their entirety). The metal particles penetrate through several layers of cells and thus allow the transformation of cells within tissue explants.

Other methods of cell transformation can also be used and include but are not limited to introduction of DNA into plants by direct DNA transfer into pollen (Zhou *et al.*, *Methods Enzymol.* 101:433 (1983); Hess *et al.*, *Intern Rev. Cytol.* 107:367 (1987); Luo *et al.*, *Plant Mol Biol. Reporter* 6:165 (1988), all of which are herein incorporated by reference in their entirety), by direct injection of DNA into reproductive organs of a plant (Pena *et al.*, *Nature* 325:274 (1987), the entirety of which is herein incorporated by reference), or by direct injection of DNA into the cells of immature embryos followed by the rehydration of desiccated embryos (Neuhaus *et al.*, *Theor. Appl. Genet.* 75:30 (1987), the entirety of which is herein incorporated by reference).

The regeneration, development and cultivation of plants from single plant protoplast transformants or from various transformed explants is well known in the art (Weissbach and Weissbach, In: *Methods for Plant Molecular Biology*, Academic Press, San Diego, CA, (1988), the entirety of which is herein incorporated by reference). This regeneration and growth process typically includes the steps of selection of transformed cells, culturing those individualized cells through the usual stages of embryonic development through the rooted plantlet stage. Transgenic embryos and seeds are similarly regenerated. The resulting transgenic rooted shoots are thereafter planted in an appropriate plant growth medium such as soil.

The development or regeneration of plants containing the foreign, exogenous gene that encodes a protein of interest is well known in the art. Preferably, the regenerated plants are self-pollinated to provide homozygous transgenic plants. Otherwise, pollen obtained from the regenerated plants is crossed to seed-grown plants of agronomically important lines. Conversely, pollen from plants of these important lines is used to pollinate regenerated plants. A transgenic plant of the present invention containing a desired polypeptide is cultivated using methods well known to one skilled in the art.

There are a variety of methods for the regeneration of plants from plant tissue. The particular method of regeneration will depend on the starting plant tissue and the particular plant species to be regenerated.

Methods for transforming dicots, primarily by use of *Agrobacterium tumefaciens* and obtaining transgenic plants have been published for cotton (U.S. Patent No. 5,004,863; U.S. Patent No. 5,159,135; U.S. Patent No. 5,518,908, all of which are herein incorporated by reference in their entirety); soybean (U.S. Patent No. 5,569,834; U.S. Patent No. 5,416,011; McCabe *et al.*, *Biotechnology* 6:923 (1988); Christou *et al.*, *Plant Physiol.* 87:671-674 (1988); all of which are herein incorporated by reference in their entirety); *Brassica* (U.S. Patent No. 5,463,174, the entirety of which is herein incorporated by reference); peanut (Cheng *et al.*, *Plant Cell Rep.* 15:653-657 (1996), McKently *et al.*, *Plant Cell Rep.* 14:699-703 (1995), all of which are herein incorporated by reference in their entirety); papaya; and pea (Grant *et al.*, *Plant Cell Rep.* 15:254-258 (1995), the entirety of which is herein incorporated by reference).

Transformation of monocotyledons using electroporation, particle bombardment and *Agrobacterium* have also been reported. Transformation and plant regeneration have been achieved in asparagus (Bytebier *et al.*, *Proc. Natl. Acad. Sci. (USA)* 84:5354 (1987), the entirety

of which is herein incorporated by reference); barley (Wan and Lemaux, *Plant Physiol* 104:37 (1994), the entirety of which is herein incorporated by reference); maize (Rhodes *et al.*, *Science* 240:204 (1988); Gordon-Kamm *et al.*, *Plant Cell* 2:603-618 (1990); Fromm *et al.*, *Bio/Technology* 8:833 (1990); Koziel *et al.*, *Bio/Technology* 11:194 (1993); Armstrong *et al.*, *Crop Science* 35:550-557 (1995); all of which are herein incorporated by reference in their entirety); oat (Somers *et al.*, *Bio/Technology* 10:1589 (1992), the entirety of which is herein incorporated by reference); orchard grass (Horn *et al.*, *Plant Cell Rep.* 7:469 (1988), the entirety of which is herein incorporated by reference); rice (Toriyama *et al.*, *Theor Appl. Genet.* 205:34 (1986); Part *et al.*, *Plant Mol. Biol.* 32:1135-1148 (1996); Abedinia *et al.*, *Aust. J. Plant Physiol.* 24:133-141 (1997); Zhang and Wu, *Theor. Appl. Genet.* 76:835 (1988); Zhang *et al.*, *Plant Cell Rep.* 7:379 (1988); Battraw and Hall, *Plant Sci.* 86:191-202 (1992); Christou *et al.*, *Bio/Technology* 9:957 (1991), all of which are herein incorporated by reference in their entirety); rye (De la Pena *et al.*, *Nature* 325:274 (1987), the entirety of which is herein incorporated by reference); sugarcane (Bower and Birch, *Plant J.* 2:409 (1992), the entirety of which is herein incorporated by reference); tall fescue (Wang *et al.*, *Bio/Technology* 10:691 (1992), the entirety of which is herein incorporated by reference) and wheat (Vasil *et al.*, *Bio/Technology* 10:667 (1992), the entirety of which is herein incorporated by reference); U.S. Patent No. 5,631,152, the entirety of which is herein incorporated by reference.)

Assays for gene expression based on the transient expression of cloned nucleic acid constructs have been developed by introducing the nucleic acid molecules into plant cells by polyethylene glycol treatment, electroporation, or particle bombardment (Marcotte *et al.*, *Nature* 335:454-457 (1988), the entirety of which is herein incorporated by reference; Marcotte *et al.*, *Plant Cell* 1:523-532 (1989), the entirety of which is herein incorporated by reference; McCarty

*et al.*, *Cell* 66:895-905 (1991), the entirety of which is herein incorporated by reference; Hattori *et al.*, *Genes Dev.* 6:609-618 (1992), the entirety of which is herein incorporated by reference; Goff *et al.*, *EMBO J.* 9:2517-2522 (1990), the entirety of which is herein incorporated by reference). Transient expression systems may be used to functionally dissect gene constructs (see generally, Mailga *et al.*, *Methods in Plant Molecular Biology*, Cold Spring Harbor Press (1995)).

Any of the nucleic acid molecules of the present invention may be introduced into a plant cell in a permanent or transient manner in combination with other genetic elements such as vectors, promoters, enhancers etc. Further, any of the nucleic acid molecules of the present invention may be introduced into a plant cell in a manner that allows for overexpression of the protein or fragment thereof encoded by the nucleic acid molecule.

Cosuppression is the reduction in expression levels, usually at the level of RNA, of a particular endogenous gene or gene family by the expression of a homologous sense construct that is capable of transcribing mRNA of the same strandedness as the transcript of the endogenous gene (Napoli *et al.*, *Plant Cell* 2:279-289 (1990), the entirety of which is herein incorporated by reference; van der Krol *et al.*, *Plant Cell* 2:291-299 (1990), the entirety of which is herein incorporated by reference). Cosuppression may result from stable transformation with a single copy nucleic acid molecule that is homologous to a nucleic acid sequence found with the cell (Prolls and Meyer, *Plant J.* 2:465-475 (1992), the entirety of which is herein incorporated by reference) or with multiple copies of a nucleic acid molecule that is homologous to a nucleic acid sequence found with the cell (Mittlesten *et al.*, *Mol. Gen. Genet.* 244:325-330 (1994), the entirety of which is herein incorporated by reference). Genes, even though different, linked to

homologous promoters may result in the cosuppression of the linked genes (Vaucheret, *C.R. Acad. Sci. III* 316:1471-1483 (1993), the entirety of which is herein incorporated by reference).

This technique has, for example, been applied to generate white flowers from red petunia and tomatoes that do not ripen on the vine. Up to 50% of petunia transformants that contained a sense copy of the glucoamylase (CHS) gene produced white flowers or floral sectors; this was as a result of the post-transcriptional loss of mRNA encoding CHS (Flavell, *Proc. Natl. Acad. Sci. (U.S.A.)* 91:3490-3496 (1994), the entirety of which is herein incorporated by reference); van Blokland *et al.*, *Plant J.* 6:861-877 (1994), the entirety of which is herein incorporated by reference). Cosuppression may require the coordinate transcription of the transgene and the endogenous gene and can be reset by a developmental control mechanism (Jorgensen, *Trends Biotechnol.* 8:340-344 (1990), the entirety of which is herein incorporated by reference; Meins and Kunz, In: *Gene Inactivation and Homologous Recombination in Plants*, Paszkowski (ed.), pp. 335-348, Kluwer Academic, Netherlands (1994), the entirety of which is herein incorporated by reference).

It is understood that one or more of the nucleic acids of the present invention may be introduced into a plant cell and transcribed using an appropriate promoter with such transcription resulting in the cosuppression of an endogenous methionine pathway protein.

Antisense approaches are a way of preventing or reducing gene function by targeting the genetic material (Mol *et al.*, *FEBS Lett.* 268:427-430 (1990), the entirety of which is herein incorporated by reference). The objective of the antisense approach is to use a sequence complementary to the target gene to block its expression and create a mutant cell line or organism in which the level of a single chosen protein is selectively reduced or abolished. Antisense techniques have several advantages over other 'reverse genetic' approaches. The site

of inactivation and its developmental effect can be manipulated by the choice of promoter for antisense genes or by the timing of external application or microinjection. Antisense can manipulate its specificity by selecting either unique regions of the target gene or regions where it shares homology to other related genes (Hiatt *et al.*, In: *Genetic Engineering*, Setlow (ed.), Vol. 11, New York: Plenum 49-63 (1989), the entirety of which is herein incorporated by reference).

The principle of regulation by antisense RNA is that RNA that is complementary to the target mRNA is introduced into cells, resulting in specific RNA:RNA duplexes being formed by base pairing between the antisense substrate and the target mRNA (Green *et al.*, *Annu. Rev. Biochem.* 55:569-597 (1986), the entirety of which is herein incorporated by reference). Under one embodiment, the process involves the introduction and expression of an antisense gene sequence. Such a sequence is one in which part or all of the normal gene sequences are placed under a promoter in inverted orientation so that the 'wrong' or complementary strand is transcribed into a noncoding antisense RNA that hybridizes with the target mRNA and interferes with its expression (Takayama and Inouye, *Crit. Rev. Biochem. Mol. Biol.* 25:155-184 (1990), the entirety of which is herein incorporated by reference). An antisense vector is constructed by standard procedures and introduced into cells by transformation, transfection, electroporation, microinjection, infection, etc. The type of transformation and choice of vector will determine whether expression is transient or stable. The promoter used for the antisense gene may influence the level, timing, tissue, specificity, or inducibility of the antisense inhibition.

It is understood that the activity of a methionine pathway protein in a plant cell may be reduced or depressed by growing a transformed plant cell containing a nucleic acid molecule whose non-transcribed strand encodes a methionine pathway protein or fragment thereof.

Antibodies have been expressed in plants (Hiatt *et al.*, *Nature* 342:76-78 (1989), the entirety of which is herein incorporated by reference; Conrad and Fielder, *Plant Mol. Biol.* 26:1023-1030 (1994), the entirety of which is herein incorporated by reference). Cytoplasmic expression of a scFv (single-chain Fv antibodies) has been reported to delay infection by artichoke mottled crinkle virus. Transgenic plants that express antibodies directed against endogenous proteins may exhibit a physiological effect (Philips *et al.*, *EMBO J.* 16:4489-4496 (1997), the entirety of which is herein incorporated by reference; Marion-Poll, *Trends in Plant Science* 2:447-448 (1997), the entirety of which is herein incorporated by reference). For example, expressed anti-abscisic antibodies have been reported to result in a general perturbation of seed development (Philips *et al.*, *EMBO J.* 16: 4489-4496 (1997)).

Antibodies that are catalytic may also be expressed in plants (abzymes). The principle behind abzymes is that since antibodies may be raised against many molecules, this recognition ability can be directed toward generating antibodies that bind transition states to force a chemical reaction forward (Persidas, *Nature Biotechnology* 15:1313-1315 (1997), the entirety of which is herein incorporated by reference; Baca *et al.*, *Ann. Rev. Biophys. Biomol. Struct.* 26:461-493 (1997), the entirety of which is herein incorporated by reference). The catalytic abilities of abzymes may be enhanced by site directed mutagenesis. Examples of abzymes are, for example, set forth in U.S. Patent No: 5,658,753; U.S. Patent No. 5,632,990; U.S. Patent No. 5,631,137; U.S. Patent 5,602,015; U.S. Patent No. 5,559,538; U.S. Patent No. 5,576,174; U.S. Patent No. 5,500,358; U.S. Patent 5,318,897; U.S. Patent No. 5,298,409; U.S. Patent No. 5,258,289 and U.S. Patent No. 5,194,585, all of which are herein incorporated in their entirety.

It is understood that any of the antibodies of the present invention may be expressed in plants and that such expression can result in a physiological effect. It is also understood that any of the expressed antibodies may be catalytic.

**(b) Fungal Constructs and Fungal Transformants**

The present invention also relates to a fungal recombinant vector comprising exogenous genetic material. The present invention also relates to a fungal cell comprising a fungal recombinant vector. The present invention also relates to methods for obtaining a recombinant fungal host cell comprising introducing into a fungal host cell exogenous genetic material.

Exogenous genetic material may be transferred into a fungal cell. In a preferred embodiment the exogenous genetic material includes a nucleic acid molecule of the present invention having a sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 3204 or complements thereof or fragments of either. The fungal recombinant vector may be any vector which can be conveniently subjected to recombinant DNA procedures. The choice of a vector will typically depend on the compatibility of the vector with the fungal host cell into which the vector is to be introduced. The vector may be a linear or a closed circular plasmid. The vector system may be a single vector or plasmid or two or more vectors or plasmids which together contain the total DNA to be introduced into the genome of the fungal host.

The fungal vector may be an autonomously replicating vector, *i.e.*, a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, *e.g.*, a plasmid, an extrachromosomal element, a minichromosome, or an artificial chromosome. The vector may contain any means for assuring self-replication. Alternatively, the vector may be one which, when introduced into the fungal cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. For integration,



the vector may rely on the nucleic acid sequence of the vector for stable integration of the vector into the genome by homologous or nonhomologous recombination. Alternatively, the vector may contain additional nucleic acid sequences for directing integration by homologous recombination into the genome of the fungal host. The additional nucleic acid sequences enable the vector to be integrated into the host cell genome at a precise location(s) in the chromosome(s). To increase the likelihood of integration at a precise location, there should be preferably two nucleic acid sequences which individually contain a sufficient number of nucleic acids, preferably 400bp to 1500bp, more preferably 800bp to 1000bp, which are highly homologous with the corresponding target sequence to enhance the probability of homologous recombination. These nucleic acid sequences may be any sequence that is homologous with a target sequence in the genome of the fungal host cell and, furthermore, may be non-encoding or encoding sequences.

For autonomous replication, the vector may further comprise an origin of replication enabling the vector to replicate autonomously in the host cell in question. Examples of origin of replications for use in a yeast host cell are the 2 micron origin of replication and the combination of CEN3 and ARS 1. Any origin of replication may be used which is compatible with the fungal host cell of choice.

The fungal vectors of the present invention preferably contain one or more selectable markers which permit easy selection of transformed cells. A selectable marker is a gene the product of which provides, for example biocide or viral resistance, resistance to heavy metals, prototrophy to auxotrophs and the like. The selectable marker may be selected from the group including, but not limited to, *amdS* (acetamidase), *argB* (ornithine carbamoyltransferase), *bar* (phosphinothricin acetyltransferase), *hygB* (hygromycin phosphotransferase), *niaD* (nitrate

reductase), *pyrG* (orotidine-5'-phosphate decarboxylase) and *sC* (sulfate adenylyltransferase) and *trpC* (anthranilate synthase). Preferred for use in an *Aspergillus* cell are the *amdS* and *pyrG* markers of *Aspergillus nidulans* or *Aspergillus oryzae* and the bar marker of *Streptomyces hygroscopicus*. Furthermore, selection may be accomplished by co-transformation, *e.g.*, as described in WO 91/17243, the entirety of which is herein incorporated by reference. A nucleic acid sequence of the present invention may be operably linked to a suitable promoter sequence. The promoter sequence is a nucleic acid sequence which is recognized by the fungal host cell for expression of the nucleic acid sequence. The promoter sequence contains transcription and translation control sequences which mediate the expression of the protein or fragment thereof.

A promoter may be any nucleic acid sequence which shows transcriptional activity in the fungal host cell of choice and may be obtained from genes encoding polypeptides either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of a nucleic acid construct of the invention in a filamentous fungal host are promoters obtained from the genes encoding *Aspergillus oryzae* TAKA amylase, *Rhizomucor miehei* aspartic proteinase, *Aspergillus niger* neutral alpha-amylase, *Aspergillus niger* acid stable alpha-amylase, *Aspergillus niger* or *Aspergillus awamori* glucoamylase (glaA), *Rhizomucor miehei* lipase, *Aspergillus oryzae* alkaline protease, *Aspergillus oryzae* triose phosphate isomerase, *Aspergillus nidulans* acetamidase and hybrids thereof. In a yeast host, a useful promoter is the *Saccharomyces cerevisiae* enolase (eno-1) promoter. Particularly preferred promoters are the TAKA amylase, NA2-tpi (a hybrid of the promoters from the genes encoding *Aspergillus niger* neutral alpha -amylase and *Aspergillus oryzae* triose phosphate isomerase) and glaA promoters.

A protein or fragment thereof encoding nucleic acid molecule of the present invention may also be operably linked to a terminator sequence at its 3' terminus. The terminator sequence may be native to the nucleic acid sequence encoding the protein or fragment thereof or may be obtained from foreign sources. Any terminator which is functional in the fungal host cell of choice may be used in the present invention, but particularly preferred terminators are obtained from the genes encoding *Aspergillus oryzae* TAKA amylase, *Aspergillus niger* glucoamylase, *Aspergillus nidulans* anthranilate synthase, *Aspergillus niger* alpha-glucosidase and *Saccharomyces cerevisiae* enolase.

A protein or fragment thereof encoding nucleic acid molecule of the present invention may also be operably linked to a suitable leader sequence. A leader sequence is a nontranslated region of a mRNA which is important for translation by the fungal host. The leader sequence is operably linked to the 5' terminus of the nucleic acid sequence encoding the protein or fragment thereof. The leader sequence may be native to the nucleic acid sequence encoding the protein or fragment thereof or may be obtained from foreign sources. Any leader sequence which is functional in the fungal host cell of choice may be used in the present invention, but particularly preferred leaders are obtained from the genes encoding *Aspergillus oryzae* TAKA amylase and *Aspergillus oryzae* triose phosphate isomerase.

A polyadenylation sequence may also be operably linked to the 3' terminus of the nucleic acid sequence of the present invention. The polyadenylation sequence is a sequence which when transcribed is recognized by the fungal host to add polyadenosine residues to transcribed mRNA. The polyadenylation sequence may be native to the nucleic acid sequence encoding the protein or fragment thereof or may be obtained from foreign sources. Any polyadenylation sequence which is functional in the fungal host of choice may be used in the present invention, but particularly

preferred polyadenylation sequences are obtained from the genes encoding *Aspergillus oryzae* TACA amylase, *Aspergillus niger* glucoamylase, *Aspergillus nidulans* anthranilate synthase and *Aspergillus niger* alpha-glucosidase.

To avoid the necessity of disrupting the cell to obtain the protein or fragment thereof and to minimize the amount of possible degradation of the expressed protein or fragment thereof within the cell, it is preferred that expression of the protein or fragment thereof gives rise to a product secreted outside the cell. To this end, a protein or fragment thereof of the present invention may be linked to a signal peptide linked to the amino terminus of the protein or fragment thereof. A signal peptide is an amino acid sequence which permits the secretion of the protein or fragment thereof from the fungal host into the culture medium. The signal peptide may be native to the protein or fragment thereof of the invention or may be obtained from foreign sources. The 5' end of the coding sequence of the nucleic acid sequence of the present invention may inherently contain a signal peptide coding region naturally linked in translation reading frame with the segment of the coding region which encodes the secreted protein or fragment thereof. Alternatively, the 5' end of the coding sequence may contain a signal peptide coding region which is foreign to that portion of the coding sequence which encodes the secreted protein or fragment thereof. The foreign signal peptide may be required where the coding sequence does not normally contain a signal peptide coding region. Alternatively, the foreign signal peptide may simply replace the natural signal peptide to obtain enhanced secretion of the desired protein or fragment thereof. The foreign signal peptide coding region may be obtained from a glucoamylase or an amylase gene from an *Aspergillus* species, a lipase or proteinase gene from *Rhizomucor miehei*, the gene for the alpha-factor from *Saccharomyces cerevisiae*, or the calf preprochymosin gene. An effective signal peptide for fungal host cells is the *Aspergillus*

*oryzae* TAKA amylase signal, *Aspergillus niger* neutral amylase signal, the *Rhizomucor miehei* aspartic proteinase signal, the *Humicola lanuginosus* cellulase signal, or the *Rhizomucor miehei* lipase signal. However, any signal peptide capable of permitting secretion of the protein or fragment thereof in a fungal host of choice may be used in the present invention.

A protein or fragment thereof encoding nucleic acid molecule of the present invention may also be linked to a propeptide coding region. A propeptide is an amino acid sequence found at the amino terminus of a protein or proenzyme. Cleavage of the propeptide from the proprotein yields a mature biochemically active protein. The resulting polypeptide is known as a propolypeptide or proenzyme (or a zymogen in some cases). Propolypeptides are generally inactive and can be converted to mature active polypeptides by catalytic or autocatalytic cleavage of the propeptide from the propolypeptide or proenzyme. The propeptide coding region may be native to the protein or fragment thereof or may be obtained from foreign sources. The foreign propeptide coding region may be obtained from the *Saccharomyces cerevisiae* alpha-factor gene or *Myceliophthora thermophila* laccase gene (WO 95/33836, the entirety of which is herein incorporated by reference).

The procedures used to ligate the elements described above to construct the recombinant expression vector of the present invention are well known to one skilled in the art (see, for example, Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual*, 2nd ed., Cold Spring Harbor, N.Y., (1989)).

The present invention also relates to recombinant fungal host cells produced by the methods of the present invention which are advantageously used with the recombinant vector of the present invention. The cell is preferably transformed with a vector comprising a nucleic acid sequence of the invention followed by integration of the vector into the host chromosome. The

choice of fungal host cells will to a large extent depend upon the gene encoding the protein or fragment thereof and its source. The fungal host cell may, for example, be a yeast cell or a filamentous fungal cell.

"Yeast" as used herein includes *Ascosporogenous* yeast (*Endomycetales*), *Basidiosporogenous* yeast and yeast belonging to the *Fungi Imperfecti* (*Blastomycetes*). The *Ascosporogenous* yeasts are divided into the families *Spermophthoraceae* and *Saccharomycetaceae*. The latter is comprised of four subfamilies, *Schizosaccharomycoideae* (for example, genus *Schizosaccharomyces*), *Nadsonioideae*, *Lipomycoideae* and *Saccharomycoideae* (for example, genera *Pichia*, *Kluyveromyces* and *Saccharomyces*). The *Basidiosporogenous* yeasts include the genera *Leucosporidim*, *Rhodosporeidium*, *Sporidiobolus*, *Filobasidium* and *Filobasidiella*. Yeast belonging to the *Fungi Imperfecti* are divided into two families, *Sporobolomycetaceae* (for example, genera *Sorobolomyces* and *Bullera*) and *Cryptococcaceae* (for example, genus *Candida*). Since the classification of yeast may change in the future, for the purposes of this invention, yeast shall be defined as described in Biology and Activities of Yeast (Skinner *et al.*, *Soc. App. Bacteriol. Symposium Series* No. 9, (1980), the entirety of which is herein incorporated by reference). The biology of yeast and manipulation of yeast genetics are well known in the art (*see*, for example, *Biochemistry and Genetics of Yeast*, Bacil *et al.* (ed.), 2nd edition, 1987; *The Yeasts*, Rose and Harrison (eds.), 2nd ed., (1987); and *The Molecular Biology of the Yeast Saccharomyces*, Strathern *et al.* (eds.), (1981), all of which are herein incorporated by reference in their entirety).

"Fungi" as used herein includes the phyla *Ascomycota*, *Basidiomycota*, *Chytridiomycota* and *Zygomycota* (as defined by Hawksworth *et al.*, In: Ainsworth and Bisby's *Dictionary of The Fungi*, 8th edition, 1995, CAB International, University Press, Cambridge, UK; the entirety of

which is herein incorporated by reference) as well as the *Oomycota* (as cited in Hawksworth *et al.*, In: Ainsworth and Bisby's *Dictionary of The Fungi*, 8th edition, 1995, CAB International, University Press, Cambridge, UK) and all mitosporic fungi (Hawksworth *et al.*, In: Ainsworth and Bisby's *Dictionary of The Fungi*, 8th edition, 1995, CAB International, University Press, Cambridge, UK). Representative groups of *Ascomycota* include, for example, *Neurospora*, *Eupenicillium* (= *Penicillium*), *Emericella* (= *Aspergillus*), *Eurotium* (= *Aspergillus*) and the true yeasts listed above. Examples of *Basidiomycota* include mushrooms, rusts and smuts.

Representative groups of *Chytridiomycota* include, for example, *Allomyces*, *Blastocladiella*, *Coelomomyces* and aquatic fungi. Representative groups of *Oomycota* include, for example, *Saprolegniomycetous* aquatic fungi (water molds) such as *Achlya*. Examples of mitosporic fungi include *Aspergillus*, *Penicillium*, *Candida* and *Alternaria*. Representative groups of *Zygomycota* include, for example, *Rhizopus* and *Mucor*.

"Filamentous fungi" include all filamentous forms of the subdivision *Eumycota* and *Oomycota* (as defined by Hawksworth *et al.*, In: Ainsworth and Bisby's *Dictionary of The Fungi*, 8th edition, 1995, CAB International, University Press, Cambridge, UK). The filamentous fungi are characterized by a vegetative mycelium composed of chitin, cellulose, glucan, chitosan, mannan and other complex polysaccharides. Vegetative growth is by hyphal elongation and carbon catabolism is obligately aerobic. In contrast, vegetative growth by yeasts such as *Saccharomyces cerevisiae* is by budding of a unicellular thallus and carbon catabolism may be fermentative.

In one embodiment, the fungal host cell is a yeast cell. In a preferred embodiment, the yeast host cell is a cell of the species of *Candida*, *Kluyveromyces*, *Saccharomyces*, *Schizosaccharomyces*, *Pichia* and *Yarrowia*. In a preferred embodiment, the yeast host cell is a

*Saccharomyces cerevisiae* cell, a *Saccharomyces carlsbergensis*, *Saccharomyces diastaticus* cell, a *Saccharomyces douglasii* cell, a *Saccharomyces kluyveri* cell, a *Saccharomyces norbensis* cell, or a *Saccharomyces oviformis* cell. In another preferred embodiment, the yeast host cell is a *Kluyveromyces lactis* cell. In another preferred embodiment, the yeast host cell is a *Yarrowia lipolytica* cell.

In another embodiment, the fungal host cell is a filamentous fungal cell. In a preferred embodiment, the filamentous fungal host cell is a cell of the species of, but not limited to, *Acremonium*, *Aspergillus*, *Fusarium*, *Humicola*, *Myceliophthora*, *Mucor*, *Neurospora*, *Penicillium*, *Thielavia*, *Tolypocladium* and *Trichoderma*. In a preferred embodiment, the filamentous fungal host cell is an *Aspergillus* cell. In another preferred embodiment, the filamentous fungal host cell is an *Acremonium* cell. In another preferred embodiment, the filamentous fungal host cell is a *Fusarium* cell. In another preferred embodiment, the filamentous fungal host cell is a *Humicola* cell. In another preferred embodiment, the filamentous fungal host cell is a *Myceliophthora* cell. In another even preferred embodiment, the filamentous fungal host cell is a *Mucor* cell. In another preferred embodiment, the filamentous fungal host cell is a *Neurospora* cell. In another preferred embodiment, the filamentous fungal host cell is a *Penicillium* cell. In another preferred embodiment, the filamentous fungal host cell is a *Thielavia* cell. In another preferred embodiment, the filamentous fungal host cell is a *Tolypocladium* cell. In another preferred embodiment, the filamentous fungal host cell is a *Trichoderma* cell. In a preferred embodiment, the filamentous fungal host cell is an *Aspergillus oryzae* cell, an *Aspergillus niger* cell, an *Aspergillus foetidus* cell, or an *Aspergillus japonicus* cell. In another preferred embodiment, the filamentous fungal host cell is a *Fusarium oxysporum* cell or a *Fusarium graminearum* cell. In another preferred embodiment, the filamentous fungal



host cell is a *Humicola insolens* cell or a *Humicola lanuginosus* cell. In another preferred embodiment, the filamentous fungal host cell is a *Myceliophthora thermophila* cell. In a most preferred embodiment, the filamentous fungal host cell is a *Mucor miehei* cell. In a most preferred embodiment, the filamentous fungal host cell is a *Neurospora crassa* cell. In a most preferred embodiment, the filamentous fungal host cell is a *Penicillium purpurogenum* cell. In another most preferred embodiment, the filamentous fungal host cell is a *Thielavia terrestris* cell. In another most preferred embodiment, the *Trichoderma* cell is a *Trichoderma reesei* cell, a *Trichoderma viride* cell, a *Trichoderma longibrachiatum* cell, a *Trichoderma harzianum* cell, or a *Trichoderma koningii* cell. In a preferred embodiment, the fungal host cell is selected from an *A. nidulans* cell, an *A. niger* cell, an *A. oryzae* cell and an *A. sojae* cell. In a further preferred embodiment, the fungal host cell is an *A. nidulans* cell.

The recombinant fungal host cells of the present invention may further comprise one or more sequences which encode one or more factors that are advantageous in the expression of the protein or fragment thereof, for example, an activator (e.g., a trans-acting factor), a chaperone and a processing protease. The nucleic acids encoding one or more of these factors are preferably not operably linked to the nucleic acid encoding the protein or fragment thereof. An activator is a protein which activates transcription of a nucleic acid sequence encoding a polypeptide (Kudla *et al.*, *EMBO* 9:1355-1364(1990); Jarai and Buxton, *Current Genetics* 26:2238-244(1994); Verdier, *Yeast* 6:271-297(1990), all of which are herein incorporated by reference in their entirety). The nucleic acid sequence encoding an activator may be obtained from the genes encoding *Saccharomyces cerevisiae* heme activator protein 1 (hap1), *Saccharomyces cerevisiae* galactose metabolizing protein 4 (gal4) and *Aspergillus nidulans* ammonia regulation protein (areA). For further examples, see Verdier, *Yeast* 6:271-297 (1990);

MacKenzie *et al.*, *Journal of Gen. Microbiol.* 139:2295-2307 (1993), both of which are herein incorporated by reference in their entirety). A chaperone is a protein which assists another protein in folding properly (Hartl *et al.*, *TIBS* 19:20-25 (1994); Bergeron *et al.*, *TIBS* 19:124-128 (1994); Demolder *et al.*, *J. Biotechnology* 32:179-189 (1994); Craig, *Science* 260:1902-1903(1993); Gething and Sambrook, *Nature* 355:33-45 (1992); Puig and Gilbert, *J Biol. Chem.* 269:7764-7771 (1994); Wang and Tsou, *FASEB Journal* 7:1515-11157 (1993); Robinson *et al.*, *Bio/Technology* 1:381-384 (1994), all of which are herein incorporated by reference in their entirety). The nucleic acid sequence encoding a chaperone may be obtained from the genes encoding *Aspergillus oryzae* protein disulphide isomerase, *Saccharomyces cerevisiae* calnexin, *Saccharomyces cerevisiae* BiP/GRP78 and *Saccharomyces cerevisiae* Hsp70. For further examples, see Gething and Sambrook, *Nature* 355:33-45 (1992); Hartl *et al.*, *TIBS* 19:20-25 (1994). A processing protease is a protease that cleaves a propeptide to generate a mature biochemically active polypeptide (Enderlin and Ogrydziak, *Yeast* 10:67-79 (1994); Fuller *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 86:1434-1438 (1989); Julius *et al.*, *Cell* 37:1075-1089 (1984); Julius *et al.*, *Cell* 32:839-852 (1983), all of which are incorporated by reference in their entirety). The nucleic acid sequence encoding a processing protease may be obtained from the genes encoding *Aspergillus niger* Kex2, *Saccharomyces cerevisiae* dipeptidylaminopeptidase, *Saccharomyces cerevisiae* Kex2 and *Yarrowia lipolytica* dibasic processing endoprotease (xpr6). Any factor that is functional in the fungal host cell of choice may be used in the present invention.

Fungal cells may be transformed by a process involving protoplast formation, transformation of the protoplasts and regeneration of the cell wall in a manner known per se. Suitable procedures for transformation of *Aspergillus* host cells are described in EP 238 023 and

Yelton *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 81:1470-1474 (1984), both of which are herein incorporated by reference in their entirety. A suitable method of transforming *Fusarium* species is described by Malardier *et al.*, *Gene* 78:147-156 (1989), the entirety of which is herein incorporated by reference. Yeast may be transformed using the procedures described by Becker and Guarente, In: Abelson and Simon, (eds.), *Guide to Yeast Genetics and Molecular Biology, Methods Enzymol.* Volume 194, pp 182-187, Academic Press, Inc., New York; Ito *et al.*, *J. Bacteriology* 153:163 (1983); Hinnen *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 75:1920 (1978), all of which are herein incorporated by reference in their entirety.

The present invention also relates to methods of producing the protein or fragment thereof comprising culturing the recombinant fungal host cells under conditions conducive for expression of the protein or fragment thereof. The fungal cells of the present invention are cultivated in a nutrient medium suitable for production of the protein or fragment thereof using methods known in the art. For example, the cell may be cultivated by shake flask cultivation, small-scale or large-scale fermentation (including continuous, batch, fed-batch, or solid state fermentations) in laboratory or industrial fermentors performed in a suitable medium and under conditions allowing the protein or fragment thereof to be expressed and/or isolated. The cultivation takes place in a suitable nutrient medium comprising carbon and nitrogen sources and inorganic salts, using procedures known in the art (*see, e.g.*, Bennett and LaSure (eds.), *More Gene Manipulations in Fungi*, Academic Press, CA, (1991), the entirety of which is herein incorporated by reference). Suitable media are available from commercial suppliers or may be prepared according to published compositions (*e.g.*, in catalogues of the American Type Culture Collection, Manassas, VA). If the protein or fragment thereof is secreted into the nutrient

medium, a protein or fragment thereof can be recovered directly from the medium. If the protein or fragment thereof is not secreted, it is recovered from cell lysates.

The expressed protein or fragment thereof may be detected using methods known in the art that are specific for the particular protein or fragment. These detection methods may include the use of specific antibodies, formation of an enzyme product, or disappearance of an enzyme substrate. For example, if the protein or fragment thereof has enzymatic activity, an enzyme assay may be used. Alternatively, if polyclonal or monoclonal antibodies specific to the protein or fragment thereof are available, immunoassays may be employed using the antibodies to the protein or fragment thereof. The techniques of enzyme assay and immunoassay are well known to those skilled in the art.

The resulting protein or fragment thereof may be recovered by methods known in the arts. For example, the protein or fragment thereof may be recovered from the nutrient medium by conventional procedures including, but not limited to, centrifugation, filtration, extraction, spray-drying, evaporation, or precipitation. The recovered protein or fragment thereof may then be further purified by a variety of chromatographic procedures, e.g., ion exchange chromatography, gel filtration chromatography, affinity chromatography, or the like.

### **(c) Mammalian Constructs and Transformed Mammalian Cells**

The present invention also relates to methods for obtaining a recombinant mammalian host cell, comprising introducing into a mammalian host cell exogenous genetic material. The present invention also relates to a mammalian cell comprising a mammalian recombinant vector. The present invention also relates to methods for obtaining a recombinant mammalian host cell, comprising introducing into a mammalian cell exogenous genetic material.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC, Manassas, VA), such as HeLa cells, Chinese hamster ovary (CHO) cells, baby hamster kidney (BHK) cells and a number of other cell lines. Suitable promoters for mammalian cells are also known in the art and include viral promoters such as that from Simian Virus 40 (SV40) (Fiers *et al.*, *Nature* 273:113 (1978), the entirety of which is herein incorporated by reference), Rous sarcoma virus (RSV), adenovirus (ADV) and bovine papilloma virus (BPV). Mammalian cells may also require terminator sequences and poly-A addition sequences. Enhancer sequences which increase expression may also be included and sequences which promote amplification of the gene may also be desirable (for example methotrexate resistance genes).

Vectors suitable for replication in mammalian cells may include viral replicons, or sequences which insure integration of the appropriate sequences encoding HCV epitopes into the host genome. For example, another vector used to express foreign DNA is vaccinia virus. In this case, for example, a nucleic acid molecule encoding a protein or fragment thereof is inserted into the vaccinia genome. Techniques for the insertion of foreign DNA into the vaccinia virus genome are known in the art and may utilize, for example, homologous recombination. Such heterologous DNA is generally inserted into a gene which is non-essential to the virus, for example, the thymidine kinase gene (tk), which also provides a selectable marker. Plasmid vectors that greatly facilitate the construction of recombinant viruses have been described (*see*, for example, Mackett *et al.*, *J Virol.* 49:857 (1984); Chakrabarti *et al.*, *Mol. Cell. Biol.* 5:3403 (1985); Moss, In: *Gene Transfer Vectors For Mammalian Cells* (Miller and Calos, eds., Cold Spring Harbor Laboratory, N.Y., p. 10, (1987); all of which are herein incorporated by reference

in their entirety). Expression of the HCV polypeptide then occurs in cells or animals which are infected with the live recombinant vaccinia virus.

The sequence to be integrated into the mammalian sequence may be introduced into the primary host by any convenient means, which includes calcium precipitated DNA, spheroplast fusion, transformation, electroporation, biolistics, lipofection, microinjection, or other convenient means. Where an amplifiable gene is being employed, the amplifiable gene may serve as the selection marker for selecting hosts into which the amplifiable gene has been introduced. Alternatively, one may include with the amplifiable gene another marker, such as a drug resistance marker, e.g. neomycin resistance (G418 in mammalian cells), hygromycin in resistance etc., or an auxotrophy marker (HIS3, TRP1, LEU2, URA3, ADE2, LYS2, etc.) for use in yeast cells.

Depending upon the nature of the modification and associated targeting construct, various techniques may be employed for identifying targeted integration. Conveniently, the DNA may be digested with one or more restriction enzymes and the fragments probed with an appropriate DNA fragment which will identify the properly sized restriction fragment associated with integration.

One may use different promoter sequences, enhancer sequences, or other sequence which will allow for enhanced levels of expression in the expression host. Thus, one may combine an enhancer from one source, a promoter region from another source, a 5'- noncoding region upstream from the initiation methionine from the same or different source as the other sequences and the like. One may provide for an intron in the non-coding region with appropriate splice sites or for an alternative 3'- untranslated sequence or polyadenylation site. Depending upon the particular purpose of the modification, any of these sequences may be introduced, as desired.

Where selection is intended, the sequence to be integrated will have with it a marker gene, which allows for selection. The marker gene may conveniently be downstream from the target gene and may include resistance to a cytotoxic agent, e.g. antibiotics, heavy metals, or the like, resistance or susceptibility to HAT, gancyclovir, etc., complementation to an auxotrophic host, particularly by using an auxotrophic yeast as the host for the subject manipulations, or the like. The marker gene may also be on a separate DNA molecule, particularly with primary mammalian cells. Alternatively, one may screen the various transformants, due to the high efficiency of recombination in yeast, by using hybridization analysis, PCR, sequencing, or the like.

For homologous recombination, constructs can be prepared where the amplifiable gene will be flanked, normally on both sides with DNA homologous with the DNA of the target region. Depending upon the nature of the integrating DNA and the purpose of the integration, the homologous DNA will generally be within 100kb, usually 50kb, preferably about 25kb, of the transcribed region of the target gene, more preferably within 2kb of the target gene. Where modeling of the gene is intended, homology will usually be present proximal to the site of the mutation. The homologous DNA may include the 5'-upstream region outside of the transcriptional regulatory region or comprising any enhancer sequences, transcriptional initiation sequences, adjacent sequences, or the like. The homologous region may include a portion of the coding region, where the coding region may be comprised only of an open reading frame or combination of exons and introns. The homologous region may comprise all or a portion of an intron, where all or a portion of one or more exons may also be present. Alternatively, the homologous region may comprise the 3'-region, so as to comprise all or a portion of the transcriptional termination region, or the region 3' of this region. The homologous regions may

extend over all or a portion of the target gene or be outside the target gene comprising all or a portion of the transcriptional regulatory regions and/or the structural gene.

The integrating constructs may be prepared in accordance with conventional ways, where sequences may be synthesized, isolated from natural sources, manipulated, cloned, ligated, subjected to in vitro mutagenesis, primer repair, or the like. At various stages, the joined sequences may be cloned and analyzed by restriction analysis, sequencing, or the like. Usually during the preparation of a construct where various fragments are joined, the fragments, intermediate constructs and constructs will be carried on a cloning vector comprising a replication system functional in a prokaryotic host, e.g., *E. coli* and a marker for selection, e.g., biocide resistance, complementation to an auxotrophic host, etc. Other functional sequences may also be present, such as polylinkers, for ease of introduction and excision of the construct or portions thereof, or the like. A large number of cloning vectors are available such as pBR322, the pUC series, etc. These constructs may then be used for integration into the primary mammalian host.

In the case of the primary mammalian host, a replicating vector may be used. Usually, such vector will have a viral replication system, such as SV40, bovine papilloma virus, adenovirus, or the like. The linear DNA sequence vector may also have a selectable marker for identifying transfected cells. Selectable markers include the neo gene, allowing for selection with G418, the herpes tk gene for selection with HAT medium, the gpt gene with mycophenolic acid, complementation of an auxotrophic host, etc.

The vector may or may not be capable of stable maintenance in the host. Where the vector is capable of stable maintenance, the cells will be screened for homologous integration of the vector into the genome of the host, where various techniques for curing the cells may be



employed. Where the vector is not capable of stable maintenance, for example, where a temperature sensitive replication system is employed, one may change the temperature from the permissive temperature to the non-permissive temperature, so that the cells may be cured of the vector. In this case, only those cells having integration of the construct comprising the amplifiable gene and, when present, the selectable marker, will be able to survive selection.

Where a selectable marker is present, one may select for the presence of the targeting construct by means of the selectable marker. Where the selectable marker is not present, one may select for the presence of the construct by the amplifiable gene. For the neo gene or the herpes tk gene, one could employ a medium for growth of the transformants of about 0.1-1 mg/ml of G418 or may use HAT medium, respectively. Where DHFR is the amplifiable gene, the selective medium may include from about 0.01-0.5  $\mu$ M of methotrexate or be deficient in glycine-hypoxanthine-thymidine and have dialysed serum (GHT media).

The DNA can be introduced into the expression host by a variety of techniques that include calcium phosphate/DNA co-precipitates, microinjection of DNA into the nucleus, electroporation, yeast protoplast fusion with intact cells, transfection, polycations, e.g., polybrene, polyornithine, etc., or the like. The DNA may be single or double stranded DNA, linear or circular. The various techniques for transforming mammalian cells are well known (see Keown *et al.*, *Methods Enzymol.* (1989); Keown *et al.*, *Methods Enzymol.* 185:527-537 (1990); Mansour *et al.*, *Nature* 336:348-352, (1988); all of which are herein incorporated by reference in their entirety).

#### **(d) Insect Constructs and Transformed Insect Cells**

The present invention also relates to an insect recombinant vectors comprising exogenous genetic material. The present invention also relates to an insect cell comprising an insect

recombinant vector. The present invention also relates to methods for obtaining a recombinant insect host cell, comprising introducing into an insect cell exogenous genetic material.

The insect recombinant vector may be any vector which can be conveniently subjected to recombinant DNA procedures and can bring about the expression of the nucleic acid sequence. The choice of a vector will typically depend on the compatibility of the vector with the insect host cell into which the vector is to be introduced. The vector may be a linear or a closed circular plasmid. The vector system may be a single vector or plasmid or two or more vectors or plasmids which together contain the total DNA to be introduced into the genome of the insect host. In addition, the insect vector may be an expression vector. Nucleic acid molecules can be suitably inserted into a replication vector for expression in the insect cell under a suitable promoter for insect cells. Many vectors are available for this purpose and selection of the appropriate vector will depend mainly on the size of the nucleic acid molecule to be inserted into the vector and the particular host cell to be transformed with the vector. Each vector contains various components depending on its function (amplification of DNA or expression of DNA) and the particular host cell with which it is compatible. The vector components for insect cell transformation generally include, but are not limited to, one or more of the following: a signal sequence, origin of replication, one or more marker genes and an inducible promoter.

The insect vector may be an autonomously replicating vector, *i.e.*, a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, *e.g.*, a plasmid, an extrachromosomal element, a minichromosome, or an artificial chromosome. The vector may contain any means for assuring self-replication. Alternatively, the vector may be one which, when introduced into the insect cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. For integration,

the vector may rely on the nucleic acid sequence of the vector for stable integration of the vector into the genome by homologous or nonhomologous recombination. Alternatively, the vector may contain additional nucleic acid sequences for directing integration by homologous recombination into the genome of the insect host. The additional nucleic acid sequences enable the vector to be integrated into the host cell genome at a precise location(s) in the chromosome(s). To increase the likelihood of integration at a precise location, there should be preferably two nucleic acid sequences which individually contain a sufficient number of nucleic acids, preferably 400bp to 1500bp, more preferably 800bp to 1000bp, which are highly homologous with the corresponding target sequence to enhance the probability of homologous recombination. These nucleic acid sequences may be any sequence that is homologous with a target sequence in the genome of the insect host cell and, furthermore, may be non-encoding or encoding sequences.

Baculovirus expression vectors (BEVs) have become important tools for the expression of foreign genes, both for basic research and for the production of proteins with direct clinical applications in human and veterinary medicine (Doerfler, *Curr. Top. Microbiol. Immunol.* 131:51-68 (1968); Luckow and Summers, *Bio/Technology* 6:47-55 (1988a); Miller, *Annual Review of Microbiol.* 42:177-199 (1988); Summers, *Curr. Comm. Molecular Biology*, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1988); all of which are herein incorporated by reference in their entirety). BEVs are recombinant insect viruses in which the coding sequence for a chosen foreign gene has been inserted behind a baculovirus promoter in place of the viral gene, e.g., polyhedrin (Smith and Summers, U.S. Pat. No., 4,745,051, the entirety of which is incorporated herein by reference).

The use of baculovirus vectors relies upon the host cells being derived from *Lepidopteran* insects such as *Spodoptera frugiperda* or *Trichoplusia ni*. The preferred *Spodoptera frugiperda* cell line is the cell line Sf9. The *Spodoptera frugiperda* Sf9 cell line was obtained from American Type Culture Collection (Manassas, VA.) and is assigned accession number ATCC CRL 1711 (Summers and Smith, *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Ag. Exper. Station Bulletin No. 1555 (1988), the entirety of which is herein incorporated by reference). Other insect cell systems, such as the silkworm *B. mori* may also be used.

The proteins expressed by the BEVs are, therefore, synthesized, modified and transported in host cells derived from *Lepidopteran* insects. Most of the genes that have been inserted and produced in the baculovirus expression vector system have been derived from vertebrate species. Other baculovirus genes in addition to the polyhedrin promoter may be employed to advantage in a baculovirus expression system. These include immediate-early ( $\alpha$ ), delayed-early ( $\beta$ ), late ( $\gamma$ ), or very late ( $\delta$ ), according to the phase of the viral infection during which they are expressed. The expression of these genes occurs sequentially, probably as the result of a "cascade" mechanism of transcriptional regulation. (Guarino and Summers, *J. Virol.* 57:563-571 (1986); Guarino and Summers, *J. Virol.* 61:2091-2099 (1987); Guarino and Summers, *Virol.* 162:444-451 (1988); all of which are herein incorporated by reference in their entirety).

Insect recombinant vectors are useful as intermediates for the infection or transformation of insect cell systems. For example, an insect recombinant vector containing a nucleic acid molecule encoding a baculovirus transcriptional promoter followed downstream by an insect signal DNA sequence is capable of directing the secretion of the desired biologically active protein from the insect cell. The vector may utilize a baculovirus transcriptional promoter region

derived from any of the over 500 baculoviruses generally infecting insects, such as for example the Orders *Lepidoptera*, *Diptera*, *Orthoptera*, *Coleoptera* and *Hymenoptera*, including for example but not limited to the viral DNAs of *Autographa californica* MNPV, *Bombyx mori* NPV, *Trichoplusia ni* MNPV, *Rachiplusia ou* MNPV or *Galleria mellonella* MNPV, wherein said baculovirus transcriptional promoter is a baculovirus immediate-early gene IEL or IEN promoter; an immediate-early gene in combination with a baculovirus delayed-early gene promoter region selected from the group consisting of 39K and a *HindIII-k* fragment delayed-early gene; or a baculovirus late gene promoter. The immediate-early or delayed-early promoters can be enhanced with transcriptional enhancer elements. The insect signal DNA sequence may code for a signal peptide of a *Lepidopteran* adipokinetic hormone precursor or a signal peptide of the *Manduca sexta* adipokinetic hormone precursor (Summers, U.S. Patent No. 5,155,037; the entirety of which is herein incorporated by reference). Other insect signal DNA sequences include a signal peptide of the *Orthoptera Schistocerca gregaria* locust adipokinetic hormone precursor and the *Drosophila melanogaster* cuticle genes CP1, CP2, CP3 or CP4 or for an insect signal peptide having substantially a similar chemical composition and function (Summers, U.S. Patent No. 5,155,037).

Insect cells are distinctly different from animal cells. Insects have a unique life cycle and have distinct cellular properties such as the lack of intracellular plasminogen activators in which are present in vertebrate cells. Another difference is the high expression levels of protein products ranging from 1 to greater than 500 mg/liter and the ease at which cDNA can be cloned into cells (Frasier, *In Vitro Cell. Dev. Biol.* 25:225 (1989); Summers and Smith, In: *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Ag. Exper. Station Bulletin No. 1555 (1988), both of which are incorporated by reference in their entirety).

Recombinant protein expression in insect cells is achieved by viral infection or stable transformation. For viral infection, the desired gene is cloned into baculovirus at the site of the wild-type polyhedron gene (Webb and Summers, *Technique* 2:173 (1990); Bishop and Posse, *Adv. Gene Technol.* 1:55 (1990); both of which are incorporated by reference in their entirety). The polyhedron gene is a component of a protein coat in occlusions which encapsulate virus particles. Deletion or insertion in the polyhedron gene results the failure to form occlusion bodies. Occlusion negative viruses are morphologically different from occlusion positive viruses and enable one skilled in the art to identify and purify recombinant viruses.

The vectors of present invention preferably contain one or more selectable markers which permit easy selection of transformed cells. A selectable marker is a gene the product of which provides, for example biocide or viral resistance, resistance to heavy metals, prototrophy to auxotrophs and the like. Selection may be accomplished by co-transformation, *e.g.*, as described in WO 91/17243, a nucleic acid sequence of the present invention may be operably linked to a suitable promoter sequence. The promoter sequence is a nucleic acid sequence which is recognized by the insect host cell for expression of the nucleic acid sequence. The promoter sequence contains transcription and translation control sequences which mediate the expression of the protein or fragment thereof. The promoter may be any nucleic acid sequence which shows transcriptional activity in the insect host cell of choice and may be obtained from genes encoding polypeptides either homologous or heterologous to the host cell.

For example, a nucleic acid molecule encoding a protein or fragment thereof may also be operably linked to a suitable leader sequence. A leader sequence is a nontranslated region of a mRNA which is important for translation by the fungal host. The leader sequence is operably linked to the 5' terminus of the nucleic acid sequence encoding the protein or fragment thereof.

The leader sequence may be native to the nucleic acid sequence encoding the protein or fragment thereof or may be obtained from foreign sources. Any leader sequence which is functional in the insect host cell of choice may be used in the present invention.

A polyadenylation sequence may also be operably linked to the 3' terminus of the nucleic acid sequence of the present invention. The polyadenylation sequence is a sequence which when transcribed is recognized by the insect host to add polyadenosine residues to transcribed mRNA. The polyadenylation sequence may be native to the nucleic acid sequence encoding the protein or fragment thereof or may be obtained from foreign sources. Any polyadenylation sequence which is functional in the fungal host of choice may be used in the present invention.

To avoid the necessity of disrupting the cell to obtain the protein or fragment thereof and to minimize the amount of possible degradation of the expressed polypeptide within the cell, it is preferred that expression of the polypeptide gene gives rise to a product secreted outside the cell. To this end, the protein or fragment thereof of the present invention may be linked to a signal peptide linked to the amino terminus of the protein or fragment thereof. A signal peptide is an amino acid sequence which permits the secretion of the protein or fragment thereof from the insect host into the culture medium. The signal peptide may be native to the protein or fragment thereof of the invention or may be obtained from foreign sources. The 5' end of the coding sequence of the nucleic acid sequence of the present invention may inherently contain a signal peptide coding region naturally linked in translation reading frame with the segment of the coding region which encodes the secreted protein or fragment thereof.

At present, a mode of achieving secretion of a foreign gene product in insect cells is by way of the foreign gene's native signal peptide. Because the foreign genes are usually from non-insect organisms, their signal sequences may be poorly recognized by insect cells and hence,

levels of expression may be suboptimal. However, the efficiency of expression of foreign gene products seems to depend primarily on the characteristics of the foreign protein. On average, nuclear localized or non-structural proteins are most highly expressed, secreted proteins are intermediate and integral membrane proteins are the least expressed. One factor generally affecting the efficiency of the production of foreign gene products in a heterologous host system is the presence of native signal sequences (also termed presequences, targeting signals, or leader sequences) associated with the foreign gene. The signal sequence is generally coded by a DNA sequence immediately following (5' to 3') the translation start site of the desired foreign gene.

The expression dependence on the type of signal sequence associated with a gene product can be represented by the following example: If a foreign gene is inserted at a site downstream from the translational start site of the baculovirus polyhedrin gene so as to produce a fusion protein (containing the N-terminus of the polyhedrin structural gene), the fused gene is highly expressed. But less expression is achieved when a foreign gene is inserted in a baculovirus expression vector immediately following the transcriptional start site and totally replacing the polyhedrin structural gene.

Insertions into the region -50 to -1 significantly alter (reduce) steady state transcription which, in turn, reduces translation of the foreign gene product. Use of the pVL941 vector optimizes transcription of foreign genes to the level of the polyhedrin gene transcription. Even though the transcription of a foreign gene may be optimal, optimal translation may vary because of several factors involving processing: signal peptide recognition, mRNA and ribosome binding, glycosylation, disulfide bond formation, sugar processing, oligomerization, for example.

The properties of the insect signal peptide are expected to be more optimal for the efficiency of the translation process in insect cells than those from vertebrate proteins. This



phenomenon can generally be explained by the fact that proteins secreted from cells are synthesized as precursor molecules containing hydrophobic N-terminal signal peptides. The signal peptides direct transport of the select protein to its target membrane and are then cleaved by a peptidase on the membrane, such as the endoplasmic reticulum, when the protein passes through it.

Another exemplary insect signal sequence is the sequence encoding for *Drosophila* cuticle proteins such as CP1, CP2, CP3 or CP4 (Summers, U.S. Patent No. 5,278,050; the entirety of which is herein incorporated by reference). Most of a 9kb region of the *Drosophila* genome containing genes for the cuticle proteins has been sequenced. Four of the five cuticle genes contains a signal peptide coding sequence interrupted by a short intervening sequence (about 60 base pairs) at a conserved site. Conserved sequences occur in the 5' mRNA untranslated region, in the adjacent 35 base pairs of upstream flanking sequence and at -200 base pairs from the mRNA start position in each of the cuticle genes.

Standard methods of insect cell culture, cotransfection and preparation of plasmids are set forth in Summers and Smith (Summers and Smith, *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Agricultural Experiment Station Bulletin No. 1555, Texas A&M University (1987)). Procedures for the cultivation of viruses and cells are described in Volkman and Summers, *J. Virol* 19:820-832 (1975) and Volkman *et al.*, *J. Virol* 19:820-832 (1976); both of which are herein incorporated by reference in their entirety.

#### **(e) Bacterial Constructs and Transformed Bacterial Cells**

The present invention also relates to a bacterial recombinant vector comprising exogenous genetic material. The present invention also relates to a bacteria cell comprising a bacterial recombinant vector. The present invention also relates to methods for obtaining a

recombinant bacteria host cell, comprising introducing into a bacterial host cell exogenous genetic material.

The bacterial recombinant vector may be any vector which can be conveniently subjected to recombinant DNA procedures. The choice of a vector will typically depend on the compatibility of the vector with the bacterial host cell into which the vector is to be introduced. The vector may be a linear or a closed circular plasmid. The vector system may be a single vector or plasmid or two or more vectors or plasmids which together contain the total DNA to be introduced into the genome of the bacterial host. In addition, the bacterial vector may be an expression vector. Nucleic acid molecules encoding protein homologues or fragments thereof can, for example, be suitably inserted into a replicable vector for expression in the bacterium under the control of a suitable promoter for bacteria. Many vectors are available for this purpose and selection of the appropriate vector will depend mainly on the size of the nucleic acid to be inserted into the vector and the particular host cell to be transformed with the vector. Each vector contains various components depending on its function (amplification of DNA or expression of DNA) and the particular host cell with which it is compatible. The vector components for bacterial transformation generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes and an inducible promoter.

In general, plasmid vectors containing replicon and control sequences that are derived from species compatible with the host cell are used in connection with bacterial hosts. The vector ordinarily carries a replication site, as well as marking sequences that are capable of providing phenotypic selection in transformed cells. For example, *E. coli* is typically transformed using pBR322, a plasmid derived from an *E. coli* species (see, e.g., Bolivar *et al.*,

*Gene* 2:95 (1977); the entirety of which is herein incorporated by reference). pBR322 contains genes for ampicillin and tetracycline resistance and thus provides easy means for identifying transformed cells. The pBR322 plasmid, or other microbial plasmid or phage, also generally contains, or is modified to contain, promoters that can be used by the microbial organism for expression of the selectable marker genes.

Nucleic acid molecules encoding protein or fragments thereof may be expressed not only directly, but also as a fusion with another polypeptide, preferably a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the polypeptide DNA that is inserted into the vector. The heterologous signal sequence selected should be one that is recognized and processed (i.e., cleaved by a signal peptidase) by the host cell. For bacterial host cells that do not recognize and process the native polypeptide signal sequence, the signal sequence is substituted by a bacterial signal sequence selected, for example, from the group consisting of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders.

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal DNA and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria.

Expression and cloning vectors also generally contain a selection gene, also termed a selectable marker. This gene encodes a protein necessary for the survival or growth of

transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for *Bacilli*. One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous protein homologue or fragment thereof produce a protein conferring drug resistance and thus survive the selection regimen.

The expression vector for producing a protein or fragment thereof can also contain an inducible promoter that is recognized by the host bacterial organism and is operably linked to the nucleic acid encoding, for example, the nucleic acid molecule encoding the protein homologue or fragment thereof of interest. Inducible promoters suitable for use with bacterial hosts include the  $\beta$ -lactamase and lactose promoter systems (Chang *et al.*, *Nature* 275:615 (1978); Goeddel *et al.*, *Nature* 281:544 (1979); both of which are herein incorporated by reference in their entirety), the arabinose promoter system (Guzman *et al.*, *J. Bacteriol.* 174:7716-7728 (1992); the entirety of which is herein incorporated by reference), alkaline phosphatase, a tryptophan (trp) promoter system (Goeddel, *Nucleic Acids Res.* 8:4057 (1980); EP 36,776; both of which are herein incorporated by reference in their entirety) and hybrid promoters such as the tac promoter (deBoer *et al.*, *Proc. Natl. Acad. Sci. (USA)* 80:21-25 (1983); the entirety of which is herein incorporated by reference). However, other known bacterial inducible promoters are suitable (Siebenlist *et al.*, *Cell* 20:269 (1980); the entirety of which is herein incorporated by reference).

Promoters for use in bacterial systems also generally contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the polypeptide of interest. The promoter can be

removed from the bacterial source DNA by restriction enzyme digestion and inserted into the vector containing the desired DNA.

Construction of suitable vectors containing one or more of the above-listed components employs standard ligation techniques. Isolated plasmids or DNA fragments are cleaved, tailored and re-ligated in the form desired to generate the plasmids required. Examples of available bacterial expression vectors include, but are not limited to, the multifunctional *E. coli* cloning and expression vectors such as Bluescript™ (Stratagene, La Jolla, CA), in which, for example, encoding an *A. nidulans* protein homologue or fragment thereof homologue, may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of  $\beta$ -galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke and Schuster, *J. Biol. Chem.* 264:5503-5509 (1989), the entirety of which is herein incorporated by reference); and the like. pGEX vectors (Promega, Madison Wisconsin U.S.A.) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems are designed to include heparin, thrombin or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

Suitable host bacteria for a bacterial vector include archaebacteria and eubacteria, especially eubacteria and most preferably *Enterobacteriaceae*. Examples of useful bacteria include *Escherichia*, *Enterobacter*, *Azotobacter*, *Erwinia*, *Bacillus*, *Pseudomonas*, *Klebsiella*, *Proteus*, *Salmonella*, *Serratia*, *Shigella*, *Rhizobia*, *Vitreoscilla* and *Paracoccus*. Suitable *E. coli* hosts include *E. coli* W3110 (American Type Culture Collection (ATCC) 27,325, Manassas,

Virginia U.S.A.), *E. coli* 294 (ATCC 31,446), *E. coli* B and *E. coli* X1776 (ATCC 31,537).

These examples are illustrative rather than limiting. Mutant cells of any of the above-mentioned bacteria may also be employed. It is, of course, necessary to select the appropriate bacteria taking into consideration replicability of the replicon in the cells of a bacterium. For example, *E. coli*, *Serratia*, or *Salmonella* species can be suitably used as the host when well known plasmids such as pBR322, pBR325, pACYC177, or pKN410 are used to supply the replicon. *E. coli* strain W3110 is a preferred host or parent host because it is a common host strain for recombinant DNA product fermentations. Preferably, the host cell should secrete minimal amounts of proteolytic enzymes.

Host cells are transfected and preferably transformed with the above-described vectors and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Numerous methods of transfection are known to the ordinarily skilled artisan, for example, calcium phosphate and electroporation. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in section 1.82 of Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, New York: Cold Spring Harbor Laboratory Press, (1989), is generally used for bacterial cells that contain substantial cell-wall barriers. Another method for transformation employs polyethylene glycol/DMSO, as described in Chung and Miller (Chung and Miller, *Nucleic Acids Res.* 16:3580 (1988); the entirety of which is herein incorporated by reference). Yet another method is the use of the technique termed electroporation.

Bacterial cells used to produce the polypeptide of interest for purposes of this invention are cultured in suitable media in which the promoters for the nucleic acid encoding the heterologous polypeptide can be artificially induced as described generally, e.g., in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, New York: Cold Spring Harbor Laboratory Press, (1989). Examples of suitable media are given in U.S. Pat. Nos. 5,304,472 and 5,342,763; both of which are incorporated by reference in their entirety.

In addition to the above discussed procedures, practitioners are familiar with the standard resource materials which describe specific conditions and procedures for the construction, manipulation and isolation of macromolecules (e.g., DNA molecules, plasmids, etc.), generation of recombinant organisms and the screening and isolating of clones, (see for example, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press (1989); Mailga *et al.*, *Methods in Plant Molecular Biology*, Cold Spring Harbor Press (1995), the entirety of which is herein incorporated by reference; Birren *et al.*, *Genome Analysis: Analyzing DNA*, 1, Cold Spring Harbor, New York, the entirety of which is herein incorporated by reference).

#### **(f) Computer Readable Media**

The nucleotide sequence provided in SEQ ID NO: 1 through SEQ ID NO: 3204 or fragment thereof, or complement thereof, or a nucleotide sequence at least 90% identical, preferably 95%, identical even more preferably 99% or 100% identical to the sequence provided in SEQ ID NO: 1 through SEQ ID NO: 3204 or fragment thereof, or complement thereof, can be “provided” in a variety of mediums to facilitate use. Such a medium can also provide a subset thereof in a form that allows a skilled artisan to examine the sequences.

A preferred subset of nucleotide sequences are those nucleic acid sequences that encode a maize or soybean methionine adenosyltransferase enzyme or complement thereof or fragment of

either, a nucleic acid molecule that encodes a maize or soybean S-adenosylmethionine  
 decarboxylase enzyme or complement thereof or fragment of either, a nucleic acid molecule that  
 encodes a maize or soybean aspartate kinase enzyme or complement thereof or fragment of  
 either, a nucleic acid molecule that encodes a maize or soybean aspartate-semialdehyde  
 dehydrogenase enzyme or complement thereof or fragment of either, a nucleic acid molecule that  
 encodes a maize or soybean *O*-succinylhomoserine (thiol)-lyase enzyme or complement thereof  
 or fragment of either, a nucleic acid molecule that encodes a maize or soybean cystathionine  $\beta$ -  
 lyase enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes  
 a maize or soybean 5-methyltetrahydropteroyltriglutamate-homocysteine-S-methyltransferase  
 enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes a  
 maize or a soybean adenosylhomocysteinase enzyme or complement thereof or fragment of  
 either, a nucleic acid molecule that encodes a maize or a soybean cystathionine  $\beta$ -synthase  
 enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes a  
 maize or a soybean cytsathionine  $\gamma$ -lyase enzyme or complement thereof or fragment of either  
 and a nucleic acid molecule that encodes a maize or a soybean *O*-acetylhomoserine (thiol)-lyase  
 enzyme or complement thereof or fragment of either.

A further preferred subset of nucleic acid sequences is where the subset of sequences is  
 two proteins or fragments thereof, more preferably three proteins or fragments thereof and even  
 more preferable four proteins or fragments thereof, these nucleic acid sequences are selected  
 from the group that comprises a maize or soybean methionine adenosyltransferase enzyme or  
 complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or  
 soybean S-adenosylmethionine decarboxylase enzyme or complement thereof or fragment of  
 either, a nucleic acid molecule that encodes a maize or soybean aspartate kinase enzyme or



complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or soybean aspartate-semialdehyde dehydrogenase enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or soybean *O*-succinylhomoserine (thiol)-lyase enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or soybean cystathionine  $\beta$ -lyase enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or soybean 5-methyltetrahydropteroyltriglutamate-homocysteine-S-methyltransferase enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean adenosylhomocysteinase enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean cystathionine  $\beta$ -synthase enzyme or complement thereof or fragment of either, a nucleic acid molecule that encodes a maize or a soybean cytsathionine  $\gamma$ -lyase enzyme or complement thereof or fragment of either and a nucleic acid molecule that encodes a maize or a soybean *O*-acetylhomoserine (thiol)-lyase enzyme or complement thereof or fragment of either.

In one application of this embodiment, a nucleotide sequence of the present invention can be recorded on computer readable media. As used herein, "computer readable media" refers to any medium that can be read and accessed directly by a computer. Such media include, but are not limited to: magnetic storage media, such as floppy discs, hard disc, storage medium and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media. A skilled artisan can readily appreciate how any of the presently known computer readable mediums can be used to create a manufacture comprising computer readable medium having recorded thereon a nucleotide sequence of the present invention.

As used herein, "recorded" refers to a process for storing information on computer readable medium. A skilled artisan can readily adopt any of the presently known methods for recording information on computer readable medium to generate media comprising the nucleotide sequence information of the present invention. A variety of data storage structures are available to a skilled artisan for creating a computer readable medium having recorded thereon a nucleotide sequence of the present invention. The choice of the data storage structure will generally be based on the means chosen to access the stored information. In addition, a variety of data processor programs and formats can be used to store the nucleotide sequence information of the present invention on computer readable medium. The sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and Microsoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. A skilled artisan can readily adapt any number of data processor structuring formats (e.g. text file or database) in order to obtain computer readable medium having recorded thereon the nucleotide sequence information of the present invention.

By providing one or more of nucleotide sequences of the present invention, a skilled artisan can routinely access the sequence information for a variety of purposes. Computer software is publicly available which allows a skilled artisan to access sequence information provided in a computer readable medium. The examples which follow demonstrate how software which implements the BLAST (Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990), the entirety of which is herein incorporated by reference) and BLAZE (Brutlag *et al.*, *Comp. Chem.* 17:203-207 (1993), the entirety of which is herein incorporated by reference) search algorithms on a Sybase system can be used to identify open reading frames (ORFs) within the genome that

contain homology to ORFs or proteins from other organisms. Such ORFs are protein-encoding fragments within the sequences of the present invention and are useful in producing commercially important proteins such as enzymes used in amino acid biosynthesis, metabolism, transcription, translation, RNA processing, nucleic acid and a protein degradation, protein modification and DNA replication, restriction, modification, recombination and repair.

The present invention further provides systems, particularly computer-based systems, which contain the sequence information described herein. Such systems are designed to identify commercially important fragments of the nucleic acid molecule of the present invention. As used herein, "a computer-based system" refers to the hardware means, software means and data storage means used to analyze the nucleotide sequence information of the present invention. The minimum hardware means of the computer-based systems of the present invention comprises a central processing unit (CPU), input means, output means and data storage means. A skilled artisan can readily appreciate that any one of the currently available computer-based system are suitable for use in the present invention.

As indicated above, the computer-based systems of the present invention comprise a data storage means having stored therein a nucleotide sequence of the present invention and the necessary hardware means and software means for supporting and implementing a search means. As used herein, "data storage means" refers to memory that can store nucleotide sequence information of the present invention, or a memory access means which can access manufactures having recorded thereon the nucleotide sequence information of the present invention. As used herein, "search means" refers to one or more programs which are implemented on the computer-based system to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search means are used to identify fragments

or regions of the sequence of the present invention that match a particular target sequence or target motif. A variety of known algorithms are disclosed publicly and a variety of commercially available software for conducting search means are available can be used in the computer-based systems of the present invention. Examples of such software include, but are not limited to, MacPattern (EMBL), BLASTIN and BLASTIX (NCBIA). One of the available algorithms or implementing software packages for conducting homology searches can be adapted for use in the present computer-based systems.

The most preferred sequence length of a target sequence is from about 10 to 100 amino acids or from about 30 to 300 nucleotide residues. However, it is well recognized that during searches for commercially important fragments of the nucleic acid molecules of the present invention, such as sequence fragments involved in gene expression and protein processing, may be of shorter length.

As used herein, "a target structural motif," or "target motif," refers to any rationally selected sequence or combination of sequences in which the sequences the sequence(s) are chosen based on a three-dimensional configuration which is formed upon the folding of the target motif. There are a variety of target motifs known in the art. Protein target motifs include, but are not limited to, enzymatic active sites and signal sequences. Nucleic acid target motifs include, but are not limited to, promoter sequences, *cis* elements, hairpin structures and inducible expression elements (protein binding sequences).

Thus, the present invention further provides an input means for receiving a target sequence, a data storage means for storing the target sequences of the present invention sequence identified using a search means as described above and an output means for outputting the identified homologous sequences. A variety of structural formats for the input and output means

can be used to input and output information in the computer-based systems of the present invention. A preferred format for an output means ranks fragments of the sequence of the present invention by varying degrees of homology to the target sequence or target motif. Such presentation provides a skilled artisan with a ranking of sequences which contain various amounts of the target sequence or target motif and identifies the degree of homology contained in the identified fragment.

A variety of comparing means can be used to compare a target sequence or target motif with the data storage means to identify sequence fragments sequence of the present invention.

For example, implementing software which implement the BLAST and BLAZE algorithms (Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990)) can be used to identify open frames within the nucleic acid molecules of the present invention. A skilled artisan can readily recognize that any one of the publicly available homology search programs can be used as the search means for the computer-based systems of the present invention.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration and are not intended to be limiting of the present invention, unless specified.

### **Example 1**

The MONN01 cDNA library is a normalized library generated from maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) total leaf tissue at the V6 plant development stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting

at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected when the maize plant is at the 6-leaf development stage. The older, more juvenile leaves, which are in a basal position, as well as the younger, more adult leaves, which are more apical are cut at the base of the leaves. The leaves are then pooled and immediately transferred to liquid nitrogen containers in which the pooled leaves are crushed. The harvested tissue is then stored at -80°C until RNA preparation.

The SATMON001 cDNA library is generated from maize (B73, Illinois Foundation Seeds, Champaign, Illinois U.S.A.) immature tassels at the V6 plant development stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in a greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue from the maize plant is collected at the V6 stage. At that stage the tassel is an immature tassel of about 2-3 cm in length. The tassels are removed and frozen in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation.

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The SATMON003 library is generated from maize (B73 x Mo17, Illinois Foundation Seeds, Champaign, Illinois U.S.A.) roots at the V6 developmental stage. Seeds are planted at a depth of approximately 3 cm in coil into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth, the seedlings are transplanted into 10 inch pots containing the Metro 200 growing medium. Plants are watered daily before transplantation and approximately 3 times a week after transplantation. Peters 15-16-17 fertilizer is applied approximately three times per week after transplanting at a concentration of 150 ppm N. Two to three times during the life time of the plant from transplanting to flowering a total of approximately 900 mg Fe is added to each pot. Maize plants are grown in the green house in approximately 15hr day/9hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected when the maize plant is at the 6 leaf development stage. The root system is cut from maize plant and washed with water to free it from the soil. The tissue is then immediately frozen in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation.

The SATMON004 cDNA library is generated from maize (B73 x Mo17, Illinois Foundation Seeds, Champaign, Illinois U.S.A.) total leaf tissue at the V6 plant development stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is

approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected when the maize plant is at the 6-leaf development stage. The older, more juvenile leaves, which are in a basal position, as well as the younger, more adult leaves, which are more apical are cut at the base of the leaves. The leaves are then pooled and immediately transferred to liquid nitrogen containers in which the pooled leaves are crushed. The harvested tissue is then stored at -80°C until RNA preparation.

The SATMON005 cDNA library is generated from maize (B73 x Mo17, Illinois Foundation Seeds, Champaign Illinois, U.S.A.) root tissue at the V6 development stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the green house in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected when the maize plant is at the 6-leaf development stage. The root system is cut from the mature maize plant and washed with water to free it from the soil. The tissue is immediately frozen in liquid nitrogen and the harvested tissue is then stored at -80°C until RNA preparation.

The SATMON006 cDNA library is generated from maize (B73 x Mo17, Illinois Foundation Seeds, Champaign Illinois, U.S.A.) total leaf tissue at the V6 plant development



stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected when the maize plant is at the 6-leaf development stage. The older more juvenile leaves, which are in a basal position, as well as the younger more adult leaves, which are more apical are cut at the base of the leaves. The leaves are then pooled and immediately transferred to liquid nitrogen containers in which the pooled leaves are crushed. The harvested tissue is then stored at -80°C until RNA preparation.

The SATMON007 cDNA library is generated from the primary root tissue of 5 day old maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) seedlings. Seeds are planted on a moist filter paper on a covered tray that is kept in the dark until germination (one day). After germination, the trays, along with the moist paper, are moved to a greenhouse where the maize plants are grown in the greenhouse in 15 hr day/9 hr night cycles for approximately 5 days. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. The primary root tissue is collected when the seedlings are 5 days old. At this stage, the primary root (radicle) is

pushed through the coleorhiza which itself is pushed through the seed coat. The primary root, which is about 2-3 cm long, is cut and immediately frozen in liquid nitrogen and then stored at -80°C until RNA preparation.

The SATMON008 cDNA library is generated from the primary shoot (coleoptile 2-3 cm) of maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) seedlings which are approximately 5 days old. Seeds are planted on a moist filter paper on a covered tray that is kept in the dark until germination (one day). Then the trays containing the seeds are moved to a greenhouse at 15hr daytime/9 hr nighttime cycles and grown until they are 5 days post germination. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Tissue is collected when the seedlings are 5 days old. At this stage, the primary shoot (coleoptile) is pushed through the seed coat and is about 2-3 cm long. The coleoptile is dissected away from the rest of the seedling, immediately frozen in liquid nitrogen and then stored at -80°C until RNA preparation.

The SATMON009 cDNA library is generated from maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) leaves at the 8 leaf stage (V8 plant development stage). Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the green house in 15 hr day/9 hr night cycles. The daytime temperature is 80°F and the nighttime temperature is

70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected when the maize plant is at the 8-leaf development stage. The older more juvenile leaves, which are in a basal position, as well as the younger more adult leaves, which are more apical, are cut at the base of the leaves. The leaves are then pooled and then immediately transferred to liquid nitrogen containers in which the pooled leaves are crushed. The harvested tissue is then stored at -80°C until RNA preparation.

The SATMON010 cDNA library is generated from maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) root tissue at the V8 plant development stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the green house in 15 hr day/9 hr night cycles. The daytime temperature is 80°F and the nighttime temperature is 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected when the maize plant is at the V8 development stage. The root system is cut from this mature maize plant and washed with water to free it from the soil. The tissue is immediately frozen in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation.

The SATMON011 cDNA library is generated from undeveloped maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) leaf at the V6 plant development stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium.

After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the green house in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected when the maize plant is at the 6-leaf development stage. The second youngest leaf which is at the base of the apical leaf of V6 stage maize plant is cut at the base and immediately transferred to liquid nitrogen containers in which the leaf is crushed. The harvested tissue is then stored at -80°C until RNA preparation.

The SATMON012 cDNA library is generated from 2 day post germination maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) seedlings. Seeds are planted on a moist filter paper on a covered tray that is kept in the dark until germination (one day). Then the trays containing the seeds are moved to the greenhouse and grown at 15hr daytime/9 hr nighttime cycles until 2 days post germination. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Tissue is collected when the seedlings are 2 days old. At the two day stage, the coleorhiza is pushed through the seed coat and the primary root (the radicle) is pierced the coleorhiza but is barely visible. Also, at this two day stage, the coleoptile is just emerging from the seed coat. The 2 days post germination seedlings are then immersed in liquid nitrogen and crushed. The harvested tissue is stored at -80°C until preparation of total RNA.

The SATMON013 cDNA library is generated from apical maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) meristem founder at the V4 plant development stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Prior to tissue collection, the plant is at the 4 leaf stage. The lead at the apex of the V4 stage maize plant is referred to as the meristem founder. This apical meristem founder is cut, immediately frozen in liquid nitrogen and crushed. The harvested tissue is then stored at -80°C until RNA preparation.

The SATMON014 cDNA library is generated from maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) endosperm fourteen days after pollination. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the greenhouse in 15 hr day/9

hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. After the V10 stage, the maize plant ear shoots are ready for fertilization. At this stage, the ear shoots are enclosed in a paper bag before silk emergence to withhold the pollen. The ear shoots are pollinated and 14 days after pollination, the ears are pulled out and then the kernels are plucked out of the ears. Each kernel is then dissected into the embryo and the endosperm and the aleurone layer is removed. After dissection, the endosperms are immediately frozen in liquid nitrogen and then stored at -80°C until RNA preparation.

The SATMON016 library is a maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) sheath library collected at the V8 developmental stage. Seeds are planted in a depth of approximately 3 cm in solid into 2-3 inch pots containing Metro growing medium. After 2-3 weeks growth, they are transplanted into 10" pots containing the same. Plants are watered daily before transplantation and approximately the times a week after transplantation. Peters 15-16-17 fertilizer is applied approximately three times per week after transplanting, at a strength of 150 ppm N. Two to three times during the life time of the plant from transplanting to flowering, a total of approximately 900 mg Fe is added to each pot. Maize plants are grown in the green house in 15hr day/9hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. When the maize plants are at the V8 stage the 5<sup>th</sup> and 6<sup>th</sup> leaves from the bottom exhibit fully developed leaf blades. At the base of these leaves, the ligule is differentiated and the leaf blade is joined to the sheath. The sheath is dissected away from the

base of the leaf then the sheath is frozen in liquid nitrogen and crushed. The tissue is then stored at -80°C until RNA preparation.

The SATMON017 cDNA library is generated from maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) embryo seventeen days after pollination. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth the seeds are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the green house in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. After the V10 stage, the ear shoots of maize plant, which are ready for fertilization, are enclosed in a paper bag before silk emergence to withhold the pollen. The ear shoots are fertilized and 21 days after pollination, the ears are pulled out and the kernels are plucked out of the ears. Each kernel is then dissected into the embryo and the endosperm and the aleurone layer is removed. After dissection, the embryos are immediately frozen in liquid nitrogen and then stored at -80°C until RNA preparation.

The SATMON019 (Lib3054) cDNA library is generated from maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) culm (stem) at the V8 developmental stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing

medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the green house in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. When the maize plant is at the V8 stage, the 5th and 6th leaves from the bottom have fully developed leaf blades. The region between the nodes of the 5th and the sixth leaves from the bottom is the region of the stem that is collected. The leaves are pulled out and the sheath is also torn away from the stem. This stem tissue is completely free of any leaf and sheath tissue. The stem tissue is then frozen in liquid nitrogen and stored at -80°C until RNA preparation.

The SATMON020 cDNA library is from a maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) Hill Type II-Initiated Callus. Petri plates containing approximately 25 ml of Type II initiation media are prepared. This medium contains N6 salts and vitamins, 3% sucrose, 2.3 g/liter proline 0.1 g/liter enzymatic casein hydrolysate, 2mg/liter 2,4 – dichloro phenoxy-acetic acid (2,4, D), 15.3 mg/liter AgNO<sub>3</sub> and 0.8% bacto agar and is adjusted to pH 6.0 before autoclaving. At 9-11 days after pollination, an ear with immature embryos measuring approximately 1-2 mm in length is chosen. The husks and silks are removed and then the ear is broken into halves and placed in an autoclaved solution of Clorox/TWEEN 20 sterilizing solution. Then the ear is rinsed with deionized water. Then each embryo is extracted from the kernel. Intact embryos are placed in contact with the medium, scutellar side up). Multiple embryos are plated on each plate and the plates are incubated in the dark at 25°C. Type II



calluses are friable, can be subcultured with a spatula, frequently regenerate via somatic embryogenesis and are relatively undifferentiated. As seen in the microscope, the Tape II calluses show color ranging from translucent to light yellow and heterogeneity on with respect to embryoid structure as well as stage of embryoid development. Once Type II callus are formed, the calluses is transferred to type II callus maintenance medium without  $\text{AgNO}_3$ . Every 7-10 days, the callus is subcultured. About 4 weeks after embryo isolation the callus is removed from the plates and then frozen in liquid nitrogen. The harvested tissue is stored at  $-80^\circ\text{C}$  until RNA preparation.

The SATMON021 cDNA library is generated from the immature maize (DK604, Dekalb Genetics, Dekalb Illinois, U.S.A.) tassel at the V8 plant development stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the green house in 15 hr day/9 hr night cycles. The daytime temperature is approximately  $80^\circ\text{F}$  and the nighttime temperature is approximately  $70^\circ\text{F}$ . Supplemental lighting is provided by 1000 W sodium vapor lamps. As the maize plant enters the V8 stage, tassels which are 15-20 cm in length are collected and frozen in liquid nitrogen. The harvested tissue is stored at  $-80^\circ\text{C}$  until RNA preparation.

The SATMON022 cDNA library is generated from maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) ear (growing silks) at the V8 plant development stage. Seeds are planted

at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. *Zea mays* plants are grown in the greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected when the plant is in the V8 stage. At this stage, some immature ear shoots are visible. The immature ear shoots (approximately 1 cm in length) are pulled out, frozen in liquid nitrogen and then stored at -80°C until RNA preparation.

The SATMON23 cDNA library is generated from maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) ear (growing silk) at the V8 development stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. When the tissue is harvested at the V8 stage, the length of the ear that is harvested is about 10-15 cm and the silks are just exposed (approximately 1 inch).

The ear along with the silks is frozen in liquid nitrogen and then the tissue is stored at -80°C until RNA preparation.

The SATMON024 cDNA library is generated from the immature maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) tassel at the V9 development stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the green house in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. As a maize plant enters the V9 stage, the tassel is rapidly developing and a 37 cm tassel along with the glume, anthers and pollen is collected and frozen in liquid nitrogen. The harvested tissue is stored at -80°C until RNA preparation.

The SATMON025 cDNA library is from maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) Hill Type II-Regenerated Callus. Type II callus is grown in initiation media as described for SATMON020 and then the embryoids on the surface of the Type II callus are allowed to mature and germinate. The 1-2 gm fresh weight of the soft friable type callus containing numerous embryoids are transferred to 100 x 15 mm petri plates containing 25 ml of regeneration media. Regeneration media consists of Murashige and Skoog (MS) basal salts, modified White's vitamins (0.2 g/liter glycine and 0.5 g/liter myo-inositol and 0.8% bacto agar (6SMS0D)). The plates are then placed in the dark after covering with parafilm. After 1 week,

the plates are moved to a lighted growth chamber with 16 hr light and 8 hr dark photoperiod.

Three weeks after plating the Type II callus to 6SMS0D, the callus exhibit shoot formation. The callus and the shoots are transferred to fresh 6SMS0D plates for another 2 weeks. The callus and the shoots are then transferred to petri plates with reduced sucrose (3SMSOD). Upon distinct formation of a root and shoot, the newly developed green plants are then removed out with a spatula and frozen in liquid nitrogen containers. The harvested tissue is then stored at  $-80^{\circ}\text{C}$  until RNA preparation.

The SATMON026 cDNA library is generated from maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) juvenile/adult shift leaves at the V8 plant development stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the green house in 15 hr day/9 hr night cycles. The daytime temperature is approximately  $80^{\circ}\text{F}$  and the nighttime temperature is approximately  $70^{\circ}\text{F}$ . Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected when the maize plants are at the 8-leaf development stage. Leaves are founded sequentially around the meristem over weeks of time and the older, more juvenile leaves arise earlier and in a more basal position than the younger, more adult leaves, which are in a more apical position. In a V8 plant, some leaves which are in the middle portion of the plant exhibit characteristics of both juvenile as well as adult leaves. They exhibit a yellowing color

but also exhibit, in part, a green color. These leaves are termed juvenile/adult shift leaves. The juvenile/adult shift leaves (the 4th, 5th leaves from the bottom) are cut at the base, pooled and transferred to liquid nitrogen in which they are then crushed. The harvested tissue is then stored at -80°C until RNA preparation.

The SATMON027 cDNA library is generated from 6 day maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) leaves. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the Metro 200 growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. *Zea mays* plants are grown in the greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Prior to tissue collection, when the plant is at the 8-leaf stage, water is held back for six days. The older, more juvenile leaves, which are in a basal position, as well as the younger, more adult leaves, which are more apical, are all cut at the base of the leaves. All the leaves exhibit significant wilting. The leaves are then pooled and immediately transferred to liquid nitrogen containers in which the pooled leaves are then crushed. The harvested tissue is then stored at -80°C until RNA preparation.

The SATMON028 cDNA library is generated from maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) roots at the V8 developmental stage that are subject to six days water

stress. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the Metro 200 growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Prior to tissue collection, when the plant is at the 8-leaf stage, water is held back for six days. The root system is cut, shaken and washed to remove soil. Root tissue is then pooled and immediately transferred to liquid nitrogen containers in which the pooled leaves are then crushed. The harvested tissue is then stored at -80°C until RNA preparation.

The SATMON029 cDNA library is generated from maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) seedlings at the etiolated stage. Seeds are planted on a moist filter paper on a covered tray that is kept in the dark for 4 days at approximately 70°F. Tissue is collected when the seedlings are 4 days old. By 4 days, the primary root has penetrated the coleorhiza and is about 4-5 cm and the secondary lateral roots have also made their appearance. The coleoptile has also pushed through the seed coat and is about 4-5 cm long. The seedlings are frozen in liquid nitrogen and crushed. The harvested tissue is then stored at -80°C until RNA preparation.

The SATMON030 cDNA library is generated from maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) root tissue at the V4 plant development stage. Seeds are planted at a

depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium.

After 2-3 weeks growth, they are transplanted into 10 inch pots containing the same. Plants are watered daily before transplantation and approximately 3 times a week after transplantation.

Peters 15-16-17 fertilizer is applied approximately three times per week after transplanting, at a strength of 150 ppm N. Two to three times during the life time of the plant, from transplanting to flowering, a total of approximately 900 mg Fe is added to each pot. Maize plants are grown in the green house in 15hr day/9hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 sodium vapor lamps. Tissue is collected when the maize plant is at the 4 leaf development stage. The root system is cut from the mature maize plant and washed with water to free it from the soil. The tissue is then immediately frozen in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation.

The SATMON031 cDNA library is generated from the maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) leaf tissue at the V4 plant development stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the green house in 15 hr day/9 hr night cycles. The daytime temperature is 80°F and the nighttime temperature is 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected

when the maize plant is at the 4-leaf development stage. The third leaf from the bottom is cut at the base and immediately frozen in liquid nitrogen and crushed. The tissue is immediately frozen in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation.

The SATMON033 cDNA library is generated from maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) embryo tissue 13 days after pollination. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. After the V10 stage, the ear shoots of the maize plant, which are ready for fertilization, are enclosed in a paper bag before silk emergent to withhold the pollen. The ear shoots are pollinated and 13 days after pollination, the ears are pulled out and then the kernels are plucked cut of the ears. Each kernel is then dissected into the embryo and the endosperm and the aleurone layer is removed. After dissection, the embryos are immediately frozen in liquid nitrogen and then stored at -80°C until RNA preparation.

The SATMON034 cDNA library is generated from cold stressed maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) seedlings. Seeds are planted on a moist filter paper on a covered tray that is kept on at 10°C for 7 days. After 7 days, the temperature is shifted to 15°C



for one day until germination of the seed. Tissue is collected once the seedlings are 1 day old. At this point, the coleorhiza has just pushed out of the seed coat and the primary root is just making its appearance. The coleoptile has not yet pushed completely through the seed coat and is also just making its appearance. These 1 day old cold stressed seedlings are frozen in liquid nitrogen and crushed. The harvested tissue is then stored at -80°C until RNA preparation.

The SATMON~001 (Lib36, Lib83, Lib84) cDNA library is generated from maize leaves at the V8 plant development stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in a greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue from the maize plant is collected at the V8 stage. The older more juvenile leaves in a basal position as well as the younger more adult leaves which are more apical are all cut at the base, pooled and frozen in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation.

The SATMONN01 cDNA library is generated from maize (B73, Illinois Foundation Seeds, Champaign, Illinois U.S.A.) normalized immature tassels at the V6 plant development stage normalized tissue. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into

10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in a greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue from the maize plant is collected at the V6 stage. At that stage the tassel is an immature tassel of about 2-3 cm in length. The tassels are removed and frozen in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation. Single stranded and double stranded DNA representing approximately  $1 \times 10^6$  colony forming units are isolated using standard protocols. RNA, complementary to the single stranded DNA, is synthesized using the double stranded DNA as a template. Biotinylated dATP is incorporated into the RNA during the synthesis reaction. The single stranded DNA is mixed with the biotinylated RNA in a 1:10 molar ratio) and allowed to hybridize. DNA-RNA hybrids are captured on Dynabeads M280 streptavidin (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.). The dynabeads with captured hybrids are collected with a magnet. The non-hybridized single stranded molecules remaining after hybrid capture are converted to double stranded form and represent the primary normalized library.

The SATMONN04 cDNA library is generated from maize (B73 x Mo17, Illinois Foundation Seeds, Champaign, Illinois U.S.A.) normalized total leaf tissue at the V6 plant development stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10

inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected when the maize plant is at the 6-leaf development stage. The older, more juvenile leaves, which are in a basal position, as well as the younger, more adult leaves, which are more apical are cut at the base of the leaves. The leaves are then pooled and immediately transferred to liquid nitrogen containers in which the pooled leaves are crushed. The harvested tissue is then stored at -80°C until RNA preparation. Single stranded and double stranded DNA representing approximately  $1 \times 10^6$  colony forming units are isolated using standard protocols. RNA, complementary to the single stranded DNA, is synthesized using the double stranded DNA as a template. Biotinylated dATP is incorporated into the RNA during the synthesis reaction. The single stranded DNA is mixed with the biotinylated RNA in a 1:10 molar ratio) and allowed to hybridize. DNA-RNA hybrids are captured on Dynabeads M280 streptavidin (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.). The dynabeads with captured hybrids are collected with a magnet. The non-hybridized single stranded molecules remaining after hybrid capture are converted to double stranded form and represent the primary normalized library.

The SATMONN05 cDNA library is generated from maize (B73 x Mo17, Illinois Foundation Seeds, Champaign Illinois, U.S.A.) normalized root tissue at the V6 development

stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the green house in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected when the maize plant is at the 6-leaf development stage. The root system is cut from the mature maize plant and washed with water to free it from the soil. The tissue is immediately frozen in liquid nitrogen and the harvested tissue is then stored at -80°C until RNA preparation. The single stranded and double stranded DNA representing approximately  $1 \times 10^6$  colony forming units are isolated using standard protocols. RNA, complementary to the single stranded DNA, is synthesized using the double stranded DNA as a template. Biotinylated dATP is incorporated into the RNA during the synthesis reaction. The single stranded DNA is mixed with the biotinylated RNA in a 1:10 molar ratio) and allowed to hybridize. DNA-RNA hybrids are captured on Dynabeads M280 streptavidin (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.). The dynabeads with captured hybrids are collected with a magnet. The non-hybridized single stranded molecules remaining after hybrid capture are converted to double stranded form and represent the primary normalized library.

The SATMONN06 cDNA library is generated from maize (B73 x Mo17, Illinois Foundation Seeds, Champaign Illinois, U.S.A.) normalized total leaf tissue at the V6 plant development stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected when the maize plant is at the 6-leaf development stage. The older more juvenile leaves, which are in a basal position, as well as the younger more adult leaves, which are more apical are cut at the base of the leaves. The leaves are then pooled and immediately transferred to liquid nitrogen containers in which the pooled leaves are crushed. The harvested tissue is then stored at -80°C until RNA preparation. Single stranded and double stranded DNA representing approximately  $1 \times 10^6$  colony forming units are isolated using standard protocols. RNA, complementary to the single stranded DNA, is synthesized using the double stranded DNA as a template. Biotinylated dATP is incorporated into the RNA during the synthesis reaction. The single stranded DNA is mixed with the biotinylated RNA in a 1:10 molar ratio) and allowed to hybridize. DNA-RNA hybrids are captured on Dynabeads M280 streptavidin (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.). The dynabeads with captured hybrids are collected with a magnet. The non-

hybridized single stranded molecules remaining after hybrid capture are converted to double stranded form and represent the primary normalized library.

The CMZ029 (SATMON036) cDNA library is generated from maize (DK604, Dekalb Genetics, Dekalb, Illinois U.S.A.) endosperm 22 days after pollination. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the green house in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. After the V10 stage, the ear shoots of the maize plant, which are ready for fertilization, are enclosed in a paper bag before silk emergent to withhold the pollen. The ear shoots are pollinated and 22 days after pollination, the ears are pulled out and then the kernels are plucked out of the ears. Each kernel is then dissected into the embryo and the endosperm and the alurone layer is removed. After dissection, the endosperms are immediately frozen in liquid nitrogen and then stored at -80°C until RNA preparation.

The CMz030 (Lib143) cDNA library is generated from maize seedling tissue two days post germination. Seeds are planted on a moist filter paper on a covered try that is keep in the dark until germination. The trays are then moved to the bench top at 15 hr daytime/9 hr nighttime cycles for 2 days post-germination. The day time temperature is 80°F and the

nighttime temperature is 70°F. Tissue is collected when the seedlings are 2 days old. At this stage, the colehrhiza has pushed through the seed coat and the primary root (the radicle) is just piercing the colehrhiza and is barely visible. The seedlings are placed at 42°C for 1 hour. Following the heat shock treatment, the seedlings are immersed in liquid nitrogen and crushed. The harvested tissue is stored at -80° until RNA preparation.

The CMz031 (Lib148) cDNA library is generated from maize pollen tissue at the V10+ plant development stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected from V10+ stage plants. The ear shoots, which are ready for fertilization, are enclosed in a paper bag to withhold pollen. Twenty-one days after pollination, prior to removing the ears, the paper bag is shaken to collect the mature pollen. The mature pollen is immediately frozen in liquid nitrogen containers and the pollen is crushed. The harvested tissue is then stored at -80°C until RNA preparation.

The CMz033 (Lib189) cDNA library is generated from maize pooled leaf tissue. Samples are harvested from open pollinated plants. Tissue is collected from maize leaves at the

anthesis stage. The leaves are collected from 10-12 plants and frozen in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation.

The CMz034 (Lib3060) cDNA library is generated from maize mature tissue at 40 days post pollination plant development stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected from leaves located two leaves below the ear leaf. This sample represents those genes expressed during onset and early stages of leaf senescence. The leaves are pooled and immediately transferred to liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation.

The CMz036 (Lib3062) cDNA library is generated from maize husk tissue at the 8 week old plant development stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during



the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected from 8 week old plants. The husk is separated from the ear and immediately transferred to liquid nitrogen containers. The harvested tissue is then stored at -80°C until RNA preparation.

The CMz037 (Lib3059) cDNA library is generated from maize pooled kernal at 12-15 days after pollination plant development stage. Sample were collected from field grown material. Whole kernal from hand pollinated (control pollination) are harvested as whole ears and immediately frozen on dry ice. Kernels from 10-12 ears were pooled and ground together in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation.

The CMz039 (Lib3066) cDNA library is generated from maize immature anther tissue at the 7 week old immature tassel stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected when the maize plant is at the 7 week old immature tassel stage. At this stage, prior to anthesis, the

immature anthers are green and enclosed in the staminate spikelet. The developing anthers are dissected away from the 7 week old immature tassel and immediately frozen in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation.

The CMz040 (Lib3067) cDNA library is generated from maize kernel tissue at the V10+ plant development stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected from V10+ stage plants. The ear shoots, which are ready for fertilization, are enclosed in a paper bag before silk emergence to withhold pollen. Five to eight days after controlled pollination. The ears are pulled and the kernels removed. The kernels are immediately frozen in liquid nitrogen. The harvested kernels tissue is then stored at -80°C until RNA preparation. This sample represents gene expressed in early kernel development, during periods of cell division, amyloplast biogenesis and early carbon flow across the material to filial tissue.

The CMz041 (Lib3068) cDNA library is generated from maize pollen germinating silk tissue at the V10+ plant development stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are

transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected from V10+ stage plants when the ear shoots are ready for fertilization at the silk emergence stage. The emerging silks are pollinated with an excess of pollen under controlled pollination conditions in the green house. Eighteen hours after pollination the silks are removed from the ears and immediately frozen in liquid nitrogen containers. This sample represents genes expressed in both pollen and silk tissue early in pollination. The harvested tissue is then stored at -80°C until RNA preparation.

The CMz042 (Lib3069) cDNA library is generated from maize ear tissue excessively pollinated at the V10+ plant development stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F.

Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected from V10+ stage plants and the ear shoots which are ready for fertilization are at the silk emergence stage. The immature ears are pollinated with an excess of pollen under controlled pollination conditions. Eighteen hours post-pollination, the ears are removed and immediately transferred to liquid nitrogen containers. The harvested tissue is then stored at -80°C until RNA preparation.

The CMz044 (Lib3075) cDNA library is generated from maize microspore tissue at the V10+ plant development stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F.

Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected from immature anthers from 7 week old tassels. The immature anthers are first dissected from the 7 week old tassel with a scalpel on a glass slide covered with water. The microspores (immature pollen) are released into the water and are recovered by centrifugation. The microspore suspension is immediately frozen in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation.

The CMz045 (Lib3076) cDNA library is generated from maize immature ear megaspore tissue. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing

Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected from immature ear (megaspore) obtained from 7 week old plants. The immature ears are harvested from the 7 week old plants and are approximately 2.5 to 3 cm in length. The kernels are removed from the cob immediately frozen in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation.

The CMz047 (Lib3078) cDNA library is generated from maize CO<sub>2</sub> treated high-exposure shoot tissue at the V10+ plant development stage. RX601 maize seeds are sterilized for 1 minute with a 10% clorox solution. The seeds are rolled in germination paper, and germinated in 0.5 mM calcium sulfate solution for two days at 30°C. The seedlings are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium at a rate of 2-3 seedlings per pot. Twenty pots are placed into a high CO<sub>2</sub> environment (approximately 1000 ppm CO<sub>2</sub>). Twenty plants were grown under ambient greenhouse CO<sub>2</sub> (approximately 450 ppm CO<sub>2</sub>). Plants are watered daily before transplantation and three times a week after transplantation. Peters 20-20-20 fertilizer is also lightly applied. Maize plants are grown in the greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime

temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. At ten days post planting, the shoots from both atmosphere are frozen in liquid nitrogen and lightly ground. The roots are washed in deionized water to remove the support media and the tissue is immediately transferred to liquid nitrogen containers. The harvested tissue is then stored at -80°C until RNA preparation.

The CMz048 (Lib3079) cDNA library is generated from maize basal endosperm transfer layer tissue at the V10+ plant development stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected from V10+ maize plants. The ear shoots, which are ready for fertilization, are enclosed in a paper bag prior to silk emergence, to withhold the pollen. Kernels are harvested at 12 days post-pollination and placed on wet ice for dissection. The kernels are cross sectioned laterally, dissecting just above the pedicel region, including 1-2 mm of the lower endosperm and the basal endosperm transfer region. The pedicel and lower endosperm region containing the basal endosperm transfer layer is pooled and immediately frozen in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation.

The CMz049(Lib3088) cDNA library is generated from maize immature anther tissue at the 7 week old immature tassel stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the greenhouse in 15 hr day/9 hr night cycles. The daytime temperature is approximately 80°F and the nighttime temperature is approximately 70°F. Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected when the maize plant is at the 7 week old immature tassel stage. At this stage, prior to anthesis, the immature anthers are green and enclosed in the staminate spikelet. The developing anthers are dissected away from the 7 week old immature tassel and immediately transferred to liquid nitrogen container. The harvested tissue is then stored at -80°C until RNA preparation.

The CMz050 (Lib3114) cDNA library is generated from maize silk tissue at the V10+ plant development stage. Seeds are planted at a depth of approximately 3 cm into 2-3 inch peat pots containing Metro 200 growing medium. After 2-3 weeks growth they are transplanted into 10 inch pots containing the same growing medium. Plants are watered daily before transplantation and three times a week after transplantation. Peters 15-16-17 fertilizer is applied three times per week after transplanting at a strength of 150 ppm N. Two to three times during the lifetime of the plant, from transplanting to flowering, a total of 900 mg Fe is added to each pot. Maize plants are grown in the greenhouse in 15 hr day/9 hr night cycles. The daytime

temperature is approximately 80°F and the nighttime temperature is approximately 70°F.

Supplemental lighting is provided by 1000 W sodium vapor lamps. Tissue is collected when the maize plant is beyond the 10-leaf development stage and the ear shoots are approximately 15-20 cm in length. The ears are pulled and silks are separated from the ears and immediately transferred to liquid nitrogen containers. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON001 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) total leaf tissue at the V4 plant development stage. Leaf tissue from 38, field grown V4 stage plants is harvested from the 4<sup>th</sup> node. Leaf tissue is removed from the plants and immediately frozen in dry-ice. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON002 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) root tissue at the V4 plant development stage. Root tissue from 76, field grown V4 stage plants is harvested. The root systems is cut from the soybean plant and washed with water to free it from the soil and immediately frozen in dry-ice. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON003 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) seedling hypocotyl axis tissue harvested 2 day post-imbibition. Seeds are planted at a depth of approximately 2cm into 2-3 inch peat pots containing Metromix 350 medium. Trays are placed in an environmental chamber and grown at 12hr daytime/12hr nighttime cycles. The daytime temperature is approximately 29°C and the nighttime temperature approximately 24°C. Soil is checked and watered daily to maintain even



moisture conditions. Tissue is collected 2 days after the start of imbibition. The 2 days after imbibition samples are separated into 3 collections after removal of any adhering seed coat. At the 2 day stage, the hypocotyl axis is emerging from the soil. A few seedlings have cracked the soil surface and exhibited slight greening of the exposed cotyledons. The seedlings are washed in water to remove soil, hypocotyl axis harvested and immediately frozen in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON004 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) seedling cotyledon tissue harvested 2 day post-imbibition. Seeds are planted at a depth of approximately 2cm into 2-3 inch peat pots containing Metromix 350 medium. Trays are placed in an environmental chamber and grown at 12hr daytime/12hr nighttime cycles. The daytime temperature is approximately 29°C and the nighttime temperature approximately 24°C. Soil is checked and watered daily to maintain even moisture conditions. Tissue is collected 2 days after the start of imbibition. The 2 days after imbibition samples are separated into 3 collections after removal of any adhering seed coat. At the 2 day stage, the hypocotyl axis is emerging from the soil. A few seedlings have cracked the soil surface and exhibited slight greening of the exposed cotyledons. The seedlings are washed in water to remove soil, hypocotyl axis harvested and immediately frozen in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON005 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) seedling hypocotyl axis tissue harvested 6 hour post-imbibition. Seeds are planted at a depth of approximately 2cm into 2-3 inch peat pots containing Metromix 350 medium. Trays are placed in an environmental chamber and grown at 12hr daytime/12hr nighttime cycles. The daytime temperature is approximately 29°C and the

nighttime temperature approximately 24°C. Soil is checked and watered daily to maintain even moisture conditions. Tissue is collected 6 hours after the start of imbibition. The 6 hours after imbibition samples are separated into 3 collections after removal of any adhering seed coat. The 6 hours after imbibition sample is collected over the course of approximately 2 hours starting at 6 hours post imbibition. At the 6 hours after imbibition stage, not all cotyledons have become fully hydrated and germination, or radicle protrusion, has not occurred. The seedlings are washed in water to remove soil, hypocotyl axis harvested and immediately frozen in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON006 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) seedling cotyledons tissue harvest 6 hour post-imbibition. Seeds are planted at a depth of approximately 2cm into 2-3 inch peat pots containing Metromix 350 medium. Trays are placed in an environmental chamber and grown at 12hr daytime/12hr nighttime cycles. The daytime temperature is approximately 29°C and the nighttime temperature approximately 24°C. Soil is checked and watered daily to maintain even moisture conditions. Tissue is collected 6 hours after imbibition. The 6 hours after imbibition samples are separated into 3 collections after removal of any adhering seed coat. The 6 hours after imbibition sample is collected over the course of approximately 2 hours starting at 6 hours post-imbibition. At the 6 hours after imbibition, not all cotyledons have become fully hydrated and germination or radicle protrusion, have not occurred. The seedlings are washed in water to remove soil, cotyledon harvested and immediately frozen in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON007 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) seed tissue harvested 25 and 35 days post-

flowering. Seed pods from field grown plants are harvested 25 and 35 days after flowering and the seeds extracted from the pods. Approximately 4.4g and 19.3g of seeds are harvested from the respective seed pods and immediately frozen in dry ice. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON008 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) leaf tissue harvested from 25 and 35 days post-flowering plants. Total leaf tissue is harvested from field grown plants. Approximately 19g and 29g of leaves are harvested from the fourth node of the plant 25 and 35 days post-flowering and immediately frozen in dry ice. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON009 cDNA library is generated from soybean cutlivar C1944 (USDA Soybean Germplasm Collection, Urbana, Illinois U.S.A.) pod and seed tissue harvested 15 days post-flowering. Pods from field grown plants are harvested 15 days post-flowering. Approximately 3g of pod tissue is harvested and immediately frozen in dry-ice. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON010 cDNA library is generated from soybean cultivar C1944 (USDA Soybean Germplasm Collection, Urbana, Illinois U.S.A.) seed tissue harvested 40 days post-flowering. Pods from field grown plants are harvested 40 days post-flowering. Pods and seeds are separated, approximately 19g of seed tissue is harvested and immediately frozen in dry-ice. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON011 cDNA library is generated from soybean cultivars Cristalina (USDA Soybean Germplasm Collection, Urbana, Illinois U.S.A.) and FT108 (Monsoy, Brazil) (tropical

germ plasma) leaf tissue. Leaves are harvested from plants grown in an environmental chamber under 12hr daytime/12hr nighttime cycles. The daytime temperature is approximately 29°C and the nighttime temperature approximately 24°C. Soil is checked and watered daily to maintain even moisture conditions. Approximately 30g of leaves are harvested from the 4<sup>th</sup> node of each of the Cristalina and FT108 cultivars and immediately frozen in dry ice. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON012 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) leaf tissue. Leaves from field grown plants are harvested from the fourth node 15 days post-flowering. Approximately 12g of leaves are harvested and immediately frozen in dry ice. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON013 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) root and nodule tissue. Approximately, 28g of root tissue from field grown plants is harvested 15 days post-flowering. The root system is cut from the soybean plant, washed with water to free it from the soil and immediately frozen in dry-ice. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON014 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) seed tissue harvested 25 and 35 days after flowering. Seed pods from field grown plants are harvested 15 days after flowering and the seeds extracted from the pods. Approximately 5g of seeds are harvested from the respective seed pods and immediately frozen in dry ice. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON015 cDNA is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) seed tissue harvested 45 and 55 days post-flowering. Seed pods from field grown plants are harvested 45 and 55 days after flowering and the seeds extracted from the pods. Approximately 19g and 31g of seeds are harvested from the respective seed pods and immediately frozen in dry ice. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON016 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) root tissue. Approximately, 61g and 38g of root tissue from field grown plants is harvested 25 and 35 days post-flowering is harvested. The root system is cut from the soybean plant and washed with water to free it from the soil. The tissue is placed in 14ml polystyrene tubes and immediately frozen in dry-ice. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON017 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) root tissue. Approximately 28g of root tissue from field grown plants is harvested 45 and 55 days post-flowering. The root system is cut from the soybean plant, washed with water to free it from the soil and immediately frozen in dry-ice. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON018 cDNA is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) leaf tissue harvested 45 and 55 days post-flowering. Leaves from field grown plants are harvested 45 and 55 days after flowering from the fourth node. Approximately 27g and 33g of seeds are harvested from the respective seed pods and immediately frozen in dry ice. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON019 cDNA library is generated from soybean cultivars Cristalina (USDA Soybean Germplasm Collection, Urbana, Illinois U.S.A.) and FT108 (Monsoy, Brazil) (tropical germ plasma) root tissue. Roots are harvested from plants grown in an environmental chamber under 12hr daytime/12hr nighttime cycles. The daytime temperature is approximately 29°C and the nighttime temperature approximately 24°C. Soil is checked and watered daily to maintain even moisture conditions. Approximately 50g and 56g of roots are harvested from each of the Cristalina and FT108 cultivars and immediately frozen in dry ice. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON020 cDNA is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) seed tissue harvested 65 and 75 days post-flowering. Seed pods from field grown plants are harvested 45 and 55 days after flowering and the seeds extracted from the pods. Approximately 14g and 31g of seeds are harvested from the respective seed pods and immediately frozen in dry ice. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON021 cDNA library is generated from Soybean Cyst Nematode-resistant soybean cultivar Hartwig (USDA Soybean Germplasm Collection, Urbana, Illinois U.S.A.) root tissue. Plants are grown in tissue culture at room temperature. At approximately 6 weeks post-germination, the plants are exposed to sterilized Soybean Cyst Nematode eggs. Infection is then allowed to progress for 10 days. After the 10 day infection process, the tissue is harvested. Agar from the culture medium and nematodes are removed and the root tissue is immediately frozen in dry ice. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON022 (Lib3030) cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) partially opened flower tissue.

Partially to fully opened flower tissue is harvested from plants grown in an environmental chamber under 12hr daytime/12hr nighttime cycles. The daytime temperature is approximately 29°C and the nighttime temperature approximately 24°C. Soil is checked and watered daily to maintain even moisture conditions. A total of 3g of flower tissue is harvested and immediately frozen in dry ice. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON023 cDNA library is generated from soybean genotype BW211S Null (Tohoku University, Morioka, Japan) seed tissue harvested 15 and 40 days post-flowering. Seed pods from field grown plants are harvested 15 and 40 days post-flowering and the seeds extracted from the pods. Approximately 0.7g and 14.2g of seeds are harvested from the respective seed pods and immediately frozen in dry ice. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON024 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) internode-2 tissue harvested 18 days post-imbibition. Seeds are planted at a depth of approximately 2cm into 2-3 inch peat pots containing Metromix 350 medium. The plants are grown in a greenhouse for 18 days after the start of imbibition at ambient temperature. Soil is checked and watered daily to maintain even moisture conditions. Stem tissue is harvested 18 days after the start of imbibition. The samples are divided into hypocotyl and internodes 1 through 5. The fifth internode contains some leaf bud material. Approximately 3 g of each sample is harvested and immediately frozen in dry ice. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON025 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) leaf tissue harvested 65 days post-flowering. Leaves are harvested from the fourth node of field grown plants 65 days post-flowering.

Approximately 18.4g of leaf tissue is harvested and immediately frozen in dry ice. The harvested tissue is then stored at -80°C until RNA preparation.

SOYMON026 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) root tissue harvested 65 and 75 days post-flowering. Approximately 27g and 40g of root tissue from field grown plants is harvested 65 and 75 days post-flowering. The root system is cut from the soybean plant, washed with water to free it from the soil and immediately frozen in dry-ice. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON027 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) seed tissue harvested 25 days post-flowering. Seed pods from field grown plants are harvested 25 days post-flowering and the seeds extracted from the pods. Approximately 17g of seeds are harvested from the seed pods and immediately frozen in dry ice. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON028 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) drought-stressed root tissue. The plants are grown in an environmental chamber under 12hr daytime/12hr nighttime cycles. The daytime temperature is approximately 29°C and the nighttime temperature 24°C. Soil is checked and watered daily to maintain even moisture conditions. At the R3 stage of development, water is withheld from half of the plant collection (drought stressed population). After 3 days, half of the plants from the drought stressed condition and half of the plants from the control population are harvested. After another 3 days (6 days post drought induction) the remaining plants are



harvested. A total of 27g and 40g of root tissue is harvested and immediately frozen in dry ice. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON029 cDNA library is generated from Soybean Cyst Nematode-resistant soybean cultivar PI07354 (USDA Soybean Germplasm Collection, Urbana, Illinois U.S.A.) root tissue. Late fall to early winter greenhouse grown plants are exposed to Soybean Cyst Nematode eggs. At 10 days post-infection, the plants are uprooted, rinsed briefly and the roots frozen in liquid nitrogen. Approximately 20 grams of root tissue is harvested from the infected plants. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON030 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) flower bud tissue. Seeds are planted at a depth of approximately 2cm into 2-3 inch peat pots containing Metromix 350 medium and the plants are grown in an environmental chamber under 12hr daytime/12hr nighttime cycles. The daytime temperature is approximately 29°C and the nighttime temperature approximately 24°C. Soil is checked and watered daily to maintain even moisture conditions. Flower buds are removed from the plant at the pedicel. A total of 100mg of flower buds are harvested and immediately frozen in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON031 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) carpel and stamen tissue. Seeds are planted at a depth of approximately 2cm into 2-3 inch peat pots containing Metromix 350 medium and the plants are grown in an environmental chamber under 12hr daytime/12hr nighttime cycles. The daytime temperature is approximately 29°C and the nighttime temperature approximately 24°C. Soil is checked and watered daily to maintain even moisture conditions. Flower buds are

removed from the plant at the pedicel. Flowers are dissected to separate petals, sepals and reproductive structures (carpels and stamens). A total of 300mg of carpel and stamen tissue are harvested and immediately frozen in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON032 cDNA library is prepared from the Asgrow cultivar A4922 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) rehydrated dry soybean seed meristem tissue. Surface sterilized seeds are germinated in liquid media for 24 hours. The seed axis is then excised from the barely germinating seed, placed on tissue culture media and incubated overnight at 20°C in the dark. The supportive tissue is removed from the explant prior to harvest. Approximately 570mg of tissue is harvested and frozen in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON033 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) heat-shocked seedling tissue without cotyledons. Seeds are imbibed and germinated in vermiculite for 2 days under constant illumination. After 48 hours, the seedlings are transferred to an incubator set at 40°C under constant illumination. After 30, 60 and 180 minutes seedlings are harvested and dissected. A portion of the seedling consisting of the root, hypocotyl and apical hook is frozen in liquid nitrogen and stored at -80°C. The seedlings after 2 days of imbibition are beginning to emerge from the vermiculite surface. The apical hooks are dark green in appearance. Total RNA and poly A<sup>+</sup> RNA is prepared from equal amounts of pooled tissue.

The SOYMON034 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) cold-shocked seedling tissue without cotyledons. Seeds are imbibed and germinated in vermiculite for 2 days under constant

illumination. After 48 hours, the seedlings are transferred to a cold room set at 5°C under constant illumination. After 30, 60 and 180 minutes seedlings are harvested and dissected. A portion of the seedling consisting of the root, hypocotyl and apical hook is frozen in liquid nitrogen and stored at -80°C. The seedlings after 2 days of imbibition are beginning to emerge from the vermiculite surface. The apical hooks are dark green in appearance.

The SOYMON035 cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) seed coat tissue. Seeds are planted at a depth of approximately 2cm into 2-3 inch peat pots containing Metromix 350 medium and the plants are grown in an environmental chamber under 12hr daytime/12hr nighttime cycles. The daytime temperature is approximately 29°C and the nighttime temperature 24°C. Soil is checked and watered daily to maintain even moisture conditions. Seeds are harvested from mid to nearly full maturation (seed coats are not yellowing). The entire embryo proper is removed from the seed coat sample and the seed coat tissue are harvested and immediately frozen in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON036 cDNA library is generated from soybean cultivars PI171451, PI227687 and PI229358 (USDA Soybean Germplasm Collection, Urbana, Illinois U.S.A.) insect challenged leaves. Plants from each of the three cultivars are grown in screenhouse conditions. The screenhouse is divided in half and one half of the screenhouse is infested with soybean looper and the other half infested with velvetbean caterpillar. A single leaf is taken from each of the representative plants at 3 different time points, 11 days after infestation, 2 weeks after infestation and 5 weeks after infestation and immediately frozen in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation. Total RNA and poly A+ RNA is

isolated from pooled tissue consisting of equal quantities of all 18 samples (3 genotypes X 3 sample times X 2 insect genotypes).

The SOYMON037 cDNA library is generated from soybean cultivar A3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) etiolated axis and radical tissue. Seeds are planted in moist vermiculite, wrapped and kept at room temperature in complete darkness until harvest. Etiolated axis and hypocotyl tissue is harvested at 2, 3 and 4 days post-planting. A total of 1 gram of each tissue type is harvested at 2, 3 and 4 days after planting and immediately frozen in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation.

The SOYMON038 cDNA library is generated from soybean variety Asgrow A3237 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) rehydrated dry seeds. Explants are prepared for transformation after germination of surface-sterilized seeds on solid tissue media. After 6 days, at 28°C and 18 hours of light per day, the germinated seeds are cold shocked at 4°C for 24 hours. Meristemic tissue and part of the hypocotyl is removed and cotyledon excised. The prepared explant is then wounded for *Agrobacterium* infection. The 2 grams of harvested tissue is frozen in liquid nitrogen and stored at -80°C until RNA preparation.

The Soy51 (LIB3027) cDNA library is prepared from equal amounts tissue harvested from SOYMON007, SOYMON015 and SOYMON020 prepared tissue. Single stranded and double stranded DNA representing approximately  $1 \times 10^6$  colony forming units are isolated using standard protocols. RNA, complementary to the single stranded DNA, is synthesized using the double stranded DNA as a template. Biotinylated dATP is incorporated into the RNA during the synthesis reaction. The single stranded DNA is mixed with the biotinylated RNA in a 1:10 molar ratio) and allowed to hybridize. DNA-RNA hybrids are captured on Dynabeads M280 streptavidin (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.). The

dynabeads with captured hybrids are collected with a magnet. The non-hybridized single stranded molecules remaining after hybrid capture are converted to double stranded form and represent the primary normalized library.

The Soy52 (LIB3028) cDNA library is generated from normalized flower DNA. Single stranded DNA representing approximately  $1 \times 10^6$  colony forming units of SOYMON022 harvested tissue is used as the starting material for normalization. RNA, complementary to the single stranded DNA, is synthesized using the double stranded DNA as a template. Biotinylated dATP is incorporated into the RNA during the synthesis reaction. The single stranded DNA is mixed with the biotinylated RNA in a 1:10 molar ratio) and allowed to hybridize. DNA-RNA hybrids are captured on Dynabeads M280 streptavidin (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.). The dynabeads with captured hybrids are collected with a magnet. The non-hybridized single stranded molecules remaining after hybrid capture are converted to double stranded form and represent the primary normalized library.

The Soy53 (LIB3039) cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) seedling shoot apical meristem tissue. Seeds are planted at a depth of approximately 2cm into 2-3 inch peat pots containing Metromix 350 medium and the plants are grown in an environmental chamber under 12hr daytime/12hr nighttime cycles. The daytime temperature is approximately 29°C and the nighttime temperature 24°C. Soil is checked and watered daily to maintain even moisture conditions. Apical tissue is harvested from seedling shoot meristem tissue, 7-8 days after the start of imbibition. The apex of each seedling is dissected to include the fifth node to the apical meristem. The fifth node corresponds to the third trifoliate leaf in the very early stages of development. Stipules completely envelop the leaf primordia at this time. A total of 200mg of apical tissue is harvested

and immediately frozen in liquid nitrogen. The harvested tissue is then stored at -80°C until RNA preparation.

The Soy54 (LIB3040) cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) heart to torpedo stage embryo tissue. Seeds are planted at a depth of approximately 2cm into 2-3 inch peat pots containing Metromix 350 medium and the plants are grown in an environmental chamber under 12hr daytime/12hr nighttime cycles. The daytime temperature is approximately 29°C and the nighttime temperature 24°C. Soil is checked and watered daily to maintain even moisture conditions. Seeds are collected and embryos removed from surrounding endosperm and maternal tissues. Embryos from globular to young torpedo stages (by corresponding analogy to *Arabidopsis*) are collected with a bias towards the middle of this spectrum. Embryos which are beginning to show asymmetric development of cotyledons are considered the upper developmental boundary for the collection and are excluded. A total of 12 mg embryo tissue is frozen in liquid nitrogen. The harvested tissue is stored at -80°C until RNA preparation.

Soy55 (LIB3049) cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) young seed tissue. Seeds are planted at a depth of approximately 2cm into 2-3 inch peat pots containing Metromix 350 medium and the plants are grown in an environmental chamber under 12hr daytime/12hr nighttime cycles. The daytime temperature is approximately 29°C and the nighttime temperature 24°C. Soil is checked and watered daily to maintain even moisture conditions. Seeds are collected from very young pods (5 to 15 days after flowering). A total of 100mg of seeds are harvested and frozen in liquid nitrogen. The harvested tissue is stored at -80°C until RNA preparation.

Soy56 (LIB3029) cDNA library is prepared from equal amounts tissue harvested from SOYMON007, SOYMON015 and SOYMON020 prepared tissue. Single stranded and double stranded DNA representing approximately  $1 \times 10^6$  colony forming units are isolated using standard protocols. RNA, complementary to the single stranded DNA, is synthesized using the double stranded DNA as a template. Biotinylated dATP is incorporated into the RNA during the synthesis reaction. The single stranded DNA is mixed with the biotinylated RNA in a 1:10 molar ratio and allowed to hybridize. DNA-RNA hybrids are captured on Dynabeads M280 streptavidin (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.). The dynabeads with captured hybrids are collected with a magnet. The non-hybridized single stranded molecules remaining after hybrid capture are not converted to double stranded form and represent a non-normalized seed pool for comparison to Soy51 cDNA libraries.

The Soy58 (LIB3050) cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) drought stressed root tissue subtracted from control root tissue. Seeds are planted at a depth of approximately 2cm into 2-3 inch peat pots containing Metromix 350 medium and the plants are grown in an environmental chamber under 12hr daytime/12hr nighttime cycles. The daytime temperature is approximately 29°C and the nighttime temperature 24°C. Soil is checked and watered daily to maintain even moisture conditions. At the R3 stage of the plant drought is induced by withholding water. After 3 and 6 days root tissue from both drought stressed and control (watered regularly) plants are collected and frozen in dry-ice. The harvested tissue is stored at -80°C until RNA preparation. For subtraction, target cDNA is made from the drought stressed tissue total RNA using the SMART cDNA synthesis system from Clontech (Clontech Laboratories, Palo Alto, California U.S.A.). Driver first strand cDNA is covalently linked to Dynabeads following a protocol similar to that

described in the Dynal literature (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.). The target cDNA is then heat denatured and the second strand trapped using Dynabeads oligo-dT. The target second strand cDNA is then hybridized to the driver cDNA in 400µl 2X SSPE for two rounds of hybridization at 65°C and 20 hours. After each hybridization, the hybridization solution is removed from the system and the hybridized target cDNA removed from the driver by heat denaturation in water. After hybridization, the remaining cDNA is trapped with Dynabeads oligo-dT. The trapped cDNA is then amplified as in previous PCR based libraries and the resulting cDNA ligated into the pSPORT vector (Invitrogen, Carlsbad California U.S.A.).

The Soy59 (LIB3051) cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) endosperm tissue. Seeds are germinated on paper towels under laboratory ambient light conditions. At 8, 10 and 14 hours after imbibition, the seed coats are harvested. The endosperm consists of a very thin layer of tissue affixed to the inside of the seed coat. The seed coat and endosperm are frozen immediately after harvest in liquid nitrogen. The harvested tissue is stored at -80°C until RNA preparation.

The Soy60 (LIB3072) cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) drought stressed seed plus pod subtracted from control seed plus pod tissue. Seeds are planted at a depth of approximately 2cm into 2-3 inch peat pots containing Metromix 350 medium and the plants are grown in an environmental chamber under 12hr daytime/12hr nighttime cycles. The daytime temperature is approximately 26°C and the nighttime temperature 21°C and 70% relative humidity. Soil is checked and watered daily to maintain even moisture conditions. At the R3 stage of the plant drought is induced by withholding water. After 3 and 6 days seeds and pods from both drought stressed and



control (watered regularly) plants are collected from the fifth and sixth node and frozen in dry-ice. The harvested tissue is stored at -80°C until RNA preparation. For subtraction, target cDNA is made from the drought stressed tissue total RNA using the SMART cDNA synthesis system from Clontech (Clontech Laboratories, Palo Alto, California U.S.A.). Driver first strand cDNA is covalently linked to Dynabeads following a protocol similar to that described in the Dynal literature (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.). The target cDNA is then heat denatured and the second strand trapped using Dynabeads oligo-dT. The target second strand cDNA is then hybridized to the driver cDNA in 400 µl 2X SSPE for two rounds of hybridization at 65°C and 20 hours. After each hybridization, the hybridization solution is removed from the system and the hybridized target cDNA removed from the driver by heat denaturation in water. After hybridization, the remaining cDNA is trapped with Dynabeads oligo-dT. The trapped cDNA is then amplified as in previous PCR based libraries and the resulting cDNA ligated into the pSPORT vector (Invitrogen, Carlsbad California U.S.A.).

The Soy61 (LIB3073) cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) jasmonic acid treated seedling subtracted from control tissue. Seeds are planted at a depth of approximately 2cm into 2-3 inch peat pots containing Metromix 350 medium and the plants are grown in a greenhouse. The daytime temperature is approximately 29.4°C and the nighttime temperature 20°C. Soil is checked and watered daily to maintain even moisture conditions. At 9 days post planting, the plantlets are sprayed with either control buffer of 0.1% Tween-20 or jasmonic acid (Sigma J-2500, Sigma, St. Louis, Missouri U.S.A.) at 1 mg/ml in 0.1% Tween-20. Plants are sprayed until runoff and the soil and the stem is soaked with the spraying solution. At 18 hours post application of jasmonic acid, the soybean plantlets appear growth retarded. After 18hours, 24hours and 48 hours post

treatment, the cotyledons are removed and the remaining leaf and stem tissue above the soil is harvested and frozen in liquid nitrogen. The harvested tissue is stored at -80°C until RNA preparation. To make RNA, the three sample timepoints were combined and ground. For subtraction, target cDNA is made from the jasmonic acid treated tissue total RNA using the SMART cDNA synthesis system from Clontech (Clontech Laboratories, Palo Alto, California U.S.A.). Driver first strand cDNA is covalently linked to Dynabeads following a protocol similar to that described in the Dynal literature (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.). The target cDNA is then heat denatured and the second strand trapped using Dynabeads oligo-dT. The target second strand cDNA is then hybridized to the driver cDNA in 400 µl 2X SSPE for two rounds of hybridization at 65°C and 20 hours. After each hybridization, the hybridization solution is removed from the system and the hybridized target cDNA removed from the driver by heat denaturation in water. After hybridization, the remaining cDNA is trapped with Dynabeads oligo-dT. The trapped cDNA is then amplified as in previous PCR based libraries and the resulting cDNA ligated into the pSPORT vector (Invitrogen, Carlsbad California U.S.A.). For this library's construction, the eighth fraction of the cDNA size fractionation step was used for ligation.

The Soy62 (LIB3074) cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) jasmonic acid treated seedling subtracted from control tissue. Seeds are planted at a depth of approximately 2cm into 2-3 inch peat pots containing Metromix 350 medium and the plants are grown in a greenhouse. The daytime temperature is approximately 29.4°C and the nighttime temperature 20°C. Soil is checked and watered daily to maintain even moisture conditions. At 9 days post planting, the plantlets are sprayed with either control buffer of 0.1% Tween-20 or jasmonic acid (Sigma J-2500, Sigma, St.

Loius, Missouri U.S.A.) at 1 mg/ml in 0.1% Tween-20. Plants are sprayed until runoff and the soil and the stem is soaked with the spraying solution. At 18 hours post application of jasmonic acid, the soybean plantlets appear growth retarded. After 18 hours, 24 hours and 48 hours post treatment, the cotyledons are removed and the remaining leaf and stem tissue above the soil is harvested and frozen in liquid nitrogen. The harvested tissue is stored at -80°C until RNA preparation. To make RNA, the three sample timepoints were combined and ground. For subtraction, target cDNA is made from the jasmonic acid treated tissue total RNA using the SMART cDNA synthesis system from Clontech (Clontech Laboratories, Palo Alto, California U.S.A.). Driver first strand cDNA is covalently linked to Dynabeads following a protocol similar to that described in the Dynal literature (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.). The target cDNA is then heat denatured and the second strand trapped using Dynabeads oligo-dT. The target second strand cDNA is then hybridized to the driver cDNA in 400 µl 2X SSPE for two rounds of hybridization at 65°C and 20 hours. After each hybridization, the hybridization solution is removed from the system and the hybridized target cDNA removed from the driver by heat denaturation in water. After hybridization, the remaining cDNA is trapped with Dynabeads oligo-dT. The trapped cDNA is then amplified as in previous PCR based libraries and the resulting cDNA ligated into the pSPORT vector (Invitrogen, Carlsbad California U.S.A.). For this library's construction, the ninth fraction of the cDNA size fractionation step was used for ligation.

The Soy65 (LIB3107) 07cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) drought-stressed abscission zone tissue. Seeds are planted at a depth of approximately 2cm into 2-3 inch peat pots containing Metromix 350 medium and the plants are grown in an environmental chamber under 12hr daytime/12hr

nighttime cycles. The daytime temperature is approximately 29°C and the nighttime temperature 24°C. Soil is checked and watered daily to maintain even moisture conditions. Plants are irrigated with 15-16-17 Peter's Mix. At the R3 stage of development, drought is imposed by withholding water. At 3, 4, 5 and 6 days, tissue is harvested and wilting is not obvious until the fourth day. Abscission layers from reproductive organs are harvested by cutting less than one millimeter proximal and distal to the layer and immediately frozen in liquid nitrogen. The harvested tissue is stored at -80°C until RNA preparation.

The Soy66 (LIB3109) cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) non-drought stressed abscission zone tissue. Seeds are planted at a depth of approximately 2cm into 2-3 inch peat pots containing Metromix 350 medium and the plants are grown in an environmental chamber under 12hr daytime/12hr nighttime cycles. The daytime temperature is approximately 29°C and the nighttime temperature approximately 24°C. Soil is checked and watered daily to maintain even moisture conditions. Plants are irrigated with 15-16-17 Peter's Mix. At 3, 4, 5 and 6 days, control abscission layer tissue is harvested. Abscission layers from reproductive organs are harvested by cutting less than one millimeter proximal and distal to the layer and immediately frozen in liquid nitrogen. The harvested tissue is stored at -80°C until RNA preparation.

Soy67 (LIB3065) cDNA library is prepared from equal amounts tissue harvested from SOYMON007, SOYMON015 and SOYMON020 prepared tissue. Single stranded and double stranded DNA representing approximately  $1 \times 10^6$  colony forming units are isolated using standard protocols. RNA, complementary to the single stranded DNA, is synthesized using the double stranded DNA as a template. Biotinylated dATP is incorporated into the RNA during the synthesis reaction. The single stranded DNA is mixed with the biotinylated RNA in a 1:10 molar

ratio) and allowed to hybridize. DNA-RNA hybrids are captured on Dynabeads M280 streptavidin (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.). The dynabeads with captured hybrids are collected with a magnet. Captured hybrids are eluted with water.

Soy68 (LIB3052) cDNA library is prepared from equal amounts tissue harvested from SOYMON007, SOYMON015 and SOYMON020 prepared tissue. Single stranded and double stranded DNA representing approximately  $1 \times 10^6$  colony forming units are isolated using standard protocols. RNA, complementary to the single stranded DNA, is synthesized using the double stranded DNA as a template. Biotinylated dATP is incorporated into the RNA during the synthesis reaction. The single stranded DNA is mixed with the biotinylated RNA in a 1:10 molar ratio) and allowed to hybridize. DNA-RNA hybrids are captured on Dynabeads M280 streptavidin (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.). The dynabeads with captured hybrids are collected with a magnet. Captured hybrids are eluted with water.

Soy69 (LIB3053) cDNA library is generated from soybean cultivars Cristalina (USDA Soybean Germplasm Collection, Urbana, Illinois U.S.A.) and FT108 (Monsoy, Brazil) (tropical germ plasma) normalized leaf tissue. Leaves are harvested from plants grown in an environmental chamber under 12hr daytime/12hr nighttime cycles. The daytime temperature is approximately 29°C and the nighttime temperature approximately 24°C. Soil is checked and watered daily to maintain even moisture conditions. Approximately 30g of leaves are harvested from the 4<sup>th</sup> node of each of the Cristalina and FT108 cultivars and immediately frozen in dry ice. The harvested tissue is then stored at -80°C until RNA preparation. Single stranded and double stranded DNA representing approximately  $1 \times 10^6$  colony forming units are isolated using standard protocols. RNA, complementary to the single stranded DNA, is synthesized using the double stranded DNA as a template. Biotinylated dATP is incorporated into the RNA during the

synthesis reaction. The single stranded DNA is mixed with the biotinylated RNA in a 1:10 molar ratio) and allowed to hybridize. DNA-RNA hybrids are captured on Dynabeads M280 streptavidin (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.). The dynabeads with captured hybrids are collected with a magnet. The non-hybridized single stranded molecules remaining after hybrid capture are converted to double stranded form and represent the primary normalized library.

Soy70 (LIB3055) cDNA library is generated from soybean cultivars Cristalina (USDA Soybean Germplasm Collection, Urbana, Illinois U.S.A.) and FT108 (Monsoy, Brazil) (tropical germ plasma) leaf tissue. Leaves are harvested from plants grown in an environmental chamber under 12hr daytime/12hr nighttime cycles. The daytime temperature is approximately 29°C and the nighttime temperature approximately 24°C. Soil is checked and watered daily to maintain even moisture conditions. Approximately 30g of leaves are harvested from the 4<sup>th</sup> node of each of the Cristalina and FT108 cultivars and immediately frozen in dry ice. The harvested tissue is then stored at -80°C until RNA preparation.

Soy71 (LIB3056) cDNA library is generated from soybean cultivars Cristalina and FT108 (tropical germ plasma) root tissue. Roots are harvested from plants grown in an environmental chamber under 12hr daytime/12hr nighttime cycles. The daytime temperature is approximately 29°C and the nighttime temperature approximately 24°C. Soil is checked and watered daily to maintain even moisture conditions. Approximately 50g and 56g of roots are harvested from each of the Cristalina and FT108 cultivars and immediately frozen in dry ice. The harvested tissue is then stored at -80°C until RNA preparation.

Soy72 (LIB3093) cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) drought stressed leaf control tissue. Seeds

are planted at a depth of approximately 2cm into 2-3 inch peat pots containing Metromix 350 medium and the plants are grown in an environmental chamber under 12hr daytime/12hr nighttime cycles. The daytime temperature is approximately 26°C and the nighttime temperature 21°C and 70% relative humidity. Soil is checked and watered daily to maintain even moisture conditions. At the R3 stage of the plant drought is induced by withholding water. After 3 and 6 days seeds and pods from both drought stressed and control (watered regularly) plants are collected from the fifth and sixth node and frozen in dry-ice. The harvested tissue is stored at -80°C until RNA preparation. For subtraction, target cDNA is made from the drought stressed tissue total RNA using the SMART cDNA synthesis system from Clontech (Clontech Laboratories, Palo Alto, California U.S.A.). Driver first strand cDNA is covalently linked to Dynabeads following a protocol similar to that described in the Dynal literature (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.). The target cDNA is then heat denatured and the second strand trapped using Dynabeads oligo-dT. The target second strand cDNA is then hybridized to the driver cDNA in 400 µl 2X SSPE for two rounds of hybridization at 65°C and 20 hours. After each hybridization, the hybridization solution is removed from the system and the hybridized target cDNA removed from the driver by heat denaturation in water. After hybridization, the remaining cDNA is trapped with Dynabeads oligo-dT. The trapped cDNA is then amplified as in previous PCR based libraries and the resulting cDNA ligated into the pSPORT vector (Invitrogen, Carlsbad California U.S.A.).

Soy73 (LIB3093) cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) drought stressed leaf subtracted from control tissue. Seeds are planted at a depth of approximately 2cm into 2-3 inch peat pots containing Metromix 350 medium and the plants are grown in an environmental chamber under

12hr daytime/12hr nighttime cycles. The daytime temperature is approximately 26°C and the nighttime temperature 21°C and 70% relative humidity. Soil is checked and watered daily to maintain even moisture conditions. At the R3 stage of the plant drought is induced by withholding water. After 3 and 6 days seeds and pods from both drought stressed and control (watered regularly) plants are collected from the fifth and sixth node and frozen in dry-ice. The harvested tissue is stored at -80°C until RNA preparation. For subtraction, target cDNA is made from the drought stressed tissue total RNA using the SMART cDNA synthesis system from Clontech (Clontech Laboratories, Palo Alto, California U.S.A.). Driver first strand cDNA is covalently linked to Dynabeads following a protocol similar to that described in the Dynal literature (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.). The target cDNA is then heat denatured and the second strand trapped using Dynabeads oligo-dT. The target second strand cDNA is then hybridized to the driver cDNA in 400 µl 2X SSPE for two rounds of hybridization at 65°C and 20 hours. After each hybridization, the hybridization solution is removed from the system and the hybridized target cDNA removed from the driver by heat denaturation in water. After hybridization, the remaining cDNA is trapped with Dynabeads oligo-dT. The trapped cDNA is then amplified as in previous PCR based libraries and the resulting cDNA ligated into the pSPORT vector (Invitrogen, Carlsbad California U.S.A.).

The Soy76 (Lib3106) cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) jasmonic acid and arachidonic treated seedling subtracted from control tissue. Seeds are planted at a depth of approximately 2cm into 2-3 inch peat pots containing Metromix 350 medium and the plants are grown in a greenhouse. The daytime temperature is approximately 29.4°C and the nighttime temperature 20°C. Soil is checked and watered daily to maintain even moisture conditions. At 9 days post planting, the



plantlets are sprayed with either control buffer of 0.1% Tween-20 or jasmonic acid (Sigma J-2500, Sigma, St. Louis, Missouri U.S.A.) at 1 mg/ml in 0.1% Tween-20. Plants are sprayed until runoff and the soil and the stem is soaked with the spraying solution. At 18 hours post application of jasmonic acid, the soybean plantlets appear growth retarded. Arachidonic treated seedlings are sprayed with 1m/ml arachidonic acid in 0.1% Tween-20. After 18hours, 24hours and 48 hours post treatment, the cotyledons are removed and the remaining leaf and stem tissue above the soil is harvested and frozen in liquid nitrogen. The harvested tissue is stored at -80°C until RNA preparation. To make RNA, the three sample timepoints were combined and ground. The RNA from the arachidonic treated seedlings is isolated separately. For subtraction, target cDNA is made from the jasmonic acid treated tissue total RNA using the SMART cDNA synthesis system from Clontech (Clontech Laboratories, Palo Alto, California U.S.A.). Driver first strand cDNA is covalently linked to Dynabeads following a protocol similar to that described in the Dynal literature (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.). The target cDNA is then heat denatured and the second strand trapped using Dynabeads oligo-dT. The target second strand cDNA is then hybridized to the driver cDNA in 400 µl 2X SSPE for two rounds of hybridization at 65°C and 20 hours. After each hybridization, the hybridization solution is removed from the system and the hybridized target cDNA removed from the driver by heat denaturation in water. After hybridization, the remaining cDNA is trapped with Dynabeads oligo-dT. The trapped cDNA is then amplified as in previous PCR based libraries and the resulting cDNA ligated into the pSPORT vector (Invitrogen, Carlsbad California U.S.A.). Fraction 10 of the size fractionated cDNA is ligated into the pSPORT vector (Invitrogen, Carlsbad California U.S.A.) in order to capture some of the smaller transcripts characteristic of antifungal proteins.

Soy77 (LIB3108) cDNA library is generated from soybean cultivar Asgrow 3244 (Asgrow Seed Company, Des Moines, Iowa U.S.A.) jasmonic acid control tissue. Seeds are planted at a depth of approximately 2cm into 2-3 inch peat pots containing Metromix 350 medium and the plants are grown in a greenhouse. The daytime temperature is approximately 29.4°C and the nighttime temperature 20°C. Soil is checked and watered daily to maintain even moisture conditions. At 9 days post planting, the plantlets are sprayed with either control buffer of 0.1% Tween-20 or jasmonic acid (Sigma J-2500, Sigma, St. Louis, Missouri U.S.A.) at 1 mg/ml in 0.1% Tween-20. Plants are sprayed until runoff and the soil and the stem is soaked with the spraying solution. At 18 hours post application of jasmonic acid, the soybean plantlets appear growth retarded. Arachidonic treated seedlings are sprayed with 1m/ml arachidonic acid in 0.1% Tween-20. After 18 hours, 24 hours and 48 hours post treatment, the cotyledons are removed and the remaining leaf and stem tissue above the soil is harvested and frozen in liquid nitrogen. The harvested tissue is stored at -80°C until RNA preparation. To make RNA, the three sample timepoints were combined and ground. The RNA from the arachidonic treated seedlings is isolated separately. For subtraction, target cDNA is made from the jasmonic acid treated tissue total RNA using the SMART cDNA synthesis system from Clontech (Clontech Laboratories, Palo Alto, California U.S.A.). Driver first strand cDNA is covalently linked to Dynabeads following a protocol similar to that described in the Dynal literature (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.). The target cDNA is then heat denatured and the second strand trapped using Dynabeads oligo-dT. The target second strand cDNA is then hybridized to the driver cDNA in 400 µl 2X SSPE for two rounds of hybridization at 65°C and 20 hours. After each hybridization, the hybridization solution is removed from the system and the hybridized target cDNA removed from the driver by heat denaturation in water. After

hybridization, the remaining cDNA is trapped with Dynabeads oligo-dT. The trapped cDNA is then amplified as in previous PCR based libraries and the resulting cDNA ligated into the pSPORT vector (Invitrogen, Carlsbad California U.S.A.). Fraction 10 of the size fractionated cDNA is ligated into the pSPORT vector in order to capture some of the smaller transcripts characteristic of antifungal proteins.

The stored RNA is purified using Trizol reagent from Life Technologies (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.), essentially as recommended by the manufacturer. Poly A<sup>+</sup> RNA (mRNA) is purified using magnetic oligo dT beads essentially as recommended by the manufacturer (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.).

Construction of plant cDNA libraries is well-known in the art and a number of cloning strategies exist. A number of cDNA library construction kits are commercially available. The Superscript™ Plasmid System for cDNA synthesis and Plasmid Cloning (Gibco BRL, Life Technologies, Gaithersburg, Maryland U.S.A.) is used, following the conditions suggested by the manufacturer.

Normalized libraries are made using essentially the Soares procedure (Soares *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 91:9228-9232 (1994), the entirety of which is herein incorporated by reference). This approach is designed to reduce the initial 10,000-fold variation in individual cDNA frequencies to achieve abundances within one order of magnitude while maintaining the overall sequence complexity of the library. In the normalization process, the prevalence of high-abundance cDNA clones decreases dramatically, clones with mid-level abundance are relatively unaffected and clones for rare transcripts are effectively increased in abundance.

## Example 2

The cDNA libraries are plated on LB agar containing the appropriate antibiotics for selection and incubated at 37° for a sufficient time to allow the growth of individual colonies. Single colonies are individually placed in each well of a 96-well microtiter plates containing LB liquid including the selective antibiotics. The plates are incubated overnight at approximately 37°C with gentle shaking to promote growth of the cultures. The plasmid DNA is isolated from each clone using Qiaprep plasmid isolation kits, using the conditions recommended by the manufacturer (Qiagen Inc., Santa Clara, California U.S.A.).

Template plasmid DNA clones are used for subsequent sequencing. For sequencing, the ABI PRISM dRhodamine Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS, is used (PE Applied Biosystems, Foster City, California U.S.A.).

## Example 3

Nucleic acid sequences that encode for the following proteins: methionine adenosyltransferase, S-adenosylmethionine decarboxylase, aspartate kinase, aspartate-semialdehyde dehydrogenase, *O*-succinylhomoserine (thiol)-lyase, cystathionine  $\beta$ -lyase, 5-methyltetrahydropteroyltriglutamate, adenosylhomocysteinase, cystathionine  $\beta$ -synthase, cystathionine  $\gamma$ -lyase and *O*-acetylhomoserine (thiol)-lyase are identified from the Monsanto EST PhytoSeq database using TBLASTN (default values)(TBLASTN compares a protein query against the six reading frames of a nucleic acid sequence). Matches found with BLAST P values equal or less than 0.001 (probability) or BLAST Score of equal or greater than 90 are classified as hits. If the program used to determine the hit is HMMSW then the score refers to HMMSW score.

In addition, the GenBank database is searched with BLASTN and BLASTX (default values) using ESTs as queries. EST that pass the hit probability threshold of  $10e^{-8}$  for the following enzymes are combined with the hits generated by using TBLASTN (described above) and classified by enzyme (see Table A below).

A cluster refers to a set of overlapping clones in the PhytoSeq database. Such an overlapping relationship among clones is designated as a “cluster” when BLAST scores from pairwise sequence comparisons of the member clones meets a predetermined minimum value or product score of 50 or more (Product Score = (BLAST SCORE x Percentage Identity)/(5 x minimum [length (Seq1), length (Seq2)]))

Since clusters are formed on the basis of single-linkage relationships, it is possible for two non-overlapping clones to be members of the same cluster if, for instance, they both overlap a third clone with at least the predetermined minimum BLAST score (stringency). A cluster ID is arbitrarily assigned to all of those clones which belong to the same cluster at a given stringency and a particular clone will belong to only one cluster at a given stringency. If a cluster contains only a single clone (a “singleton”), then the cluster ID number will be negative, with an absolute value equal to the clone ID number of its single member. Clones grouped in a cluster in most cases represent a contiguous sequence.

TABLE A\*

METHIONINE ADENOSYLTRANSFERASE ( EC 2.5.1.6 )								
Seq No.	Cluster ID	CloneID	Library	NCBI gi	Method	Score	P-value	%Ident
1	-700019427	700019427H1	SATMON001	g17262	BLASTX	120	1e-11	92
2	-700074004	700074004H1	SATMON007	g1778820	BLASTN	830	1e-60	80
3	-700149718	700149718H1	SATMON007	g1778820	BLASTN	263	1e-13	80
4	-700220251	700220251H1	SATMON011	g1127582	BLASTN	243	1e-9	90
5	-700458196	700458196H1	SATMON029	g1778820	BLASTN	202	1e-22	88
6	-701172459	701172459H1	SATMONN05	g882334	BLASTN	801	1e-57	89
7	-L1436317	LIB143-062-Q1-E1-B6	LIB143	g1778820	BLASTN	341	1e-19	77
8	-L1893126	LIB189-021-Q1-E1-F4	LIB189	g1778820	BLASTN	629	1e-41	88
9	-L1894542	LIB189-033-Q1-E1-H12	LIB189	g1778820	BLASTN	564	1e-60	76
10	-L30622839	LIB3062-028-Q1-K1-C2	LIB3062	g1778820	BLASTN	625	1e-65	74
11	-L30671966	LIB3067-036-Q1-K1-D8	LIB3067	g1655576	BLASTX	118	1e-25	85
12	-L30682166	LIB3068-004-Q1-K1-D5	LIB3068	g497900	BLASTX	111	1e-29	47
13	1	LIB143-036-Q1-E1-G7	LIB143	g450548	BLASTN	1766	1e-138	88
14	1	LIB3060-013-Q1-K1-F7	LIB3060	g1778820	BLASTN	1702	1e-133	90
15	1	LIB143-013-Q1-E1-A6	LIB143	g1778820	BLASTN	1712	1e-133	88
16	1	LIB148-007-Q1-E1-B8	LIB148	g450548	BLASTN	1693	1e-132	88
17	1	LIB3079-003-Q1-K1-G1	LIB3079	g1778820	BLASTN	1236	1e-130	88
18	1	LIB3066-011-Q1-K1-E4	LIB3066	g1778820	BLASTN	1661	1e-129	86
19	1	LIB3068-008-Q1-K1-D2	LIB3068	g450548	BLASTN	1192	1e-128	88
20	1	LIB143-061-Q1-E1-E7	LIB143	g1778820	BLASTN	1638	1e-127	88
21	1	LIB189-006-Q1-E1-B2	LIB189	g1778820	BLASTN	1627	1e-126	86
22	1	LIB143-031-Q1-E1-C7	LIB143	g450548	BLASTN	1595	1e-124	86
23	1	LIB3068-011-Q1-K1-B12	LIB3068	g450548	BLASTN	1552	1e-123	87
24	1	LIB143-013-Q1-E1-G11	LIB143	g450548	BLASTN	1585	1e-123	88
25	1	LIB3062-057-Q1-K1-H9	LIB3062	g450548	BLASTN	1577	1e-122	89
26	1	LIB143-008-Q1-E1-G9	LIB143	g450548	BLASTN	1556	1e-120	89
27	1	LIB3068-058-Q1-K1-B7	LIB3068	g450548	BLASTN	1470	1e-118	86
28	1	LIB3067-048-Q1-K1-G5	LIB3067	g1778820	BLASTN	1518	1e-117	89
29	1	LIB143-049-Q1-E1-A12	LIB143	g1778820	BLASTN	1480	1e-114	91
30	1	LIB3062-010-Q1-K1-C7	LIB3062	g1778820	BLASTN	1145	1e-113	87
31	1	LIB3068-055-Q1-K1-E10	LIB3068	g450548	BLASTN	1451	1e-112	88
32	1	LIB189-025-Q1-E1-H1	LIB189	g1778820	BLASTN	1460	1e-112	90
33	1	LIB143-066-Q1-E1-F4	LIB143	g1778820	BLASTN	1309	1e-111	86
34	1	LIB143-003-Q1-E1-D10	LIB143	g1778820	BLASTN	1440	1e-111	87
35	1	LIB3062-033-Q1-K1-G1	LIB3062	g1778820	BLASTN	1449	1e-111	86
36	1	LIB3066-029-Q1-K1-H11	LIB3066	g1778820	BLASTN	1427	1e-110	88
37	1	LIB3068-016-Q1-K1-D9	LIB3068	g450548	BLASTN	1213	1e-109	87
38	1	LIB3062-027-Q1-K1-B11	LIB3062	g1778820	BLASTN	1187	1e-108	87
39	1	LIB3068-048-Q1-K1-G4	LIB3068	g1778820	BLASTN	1410	1e-108	82
40	1	LIB148-020-Q1-E1-B6	LIB148	g1778820	BLASTN	1088	1e-107	84
41	1	LIB3066-046-Q1-K1-F2	LIB3066	g1778820	BLASTN	1400	1e-107	90
42	1	700084130H1	SATMON011	g1778820	BLASTN	960	1e-106	92
43	1	700092863H1	SATMON008	g1778820	BLASTN	1388	1e-106	92
44	1	LIB3068-043-Q1-K1-A12	LIB3068	g450548	BLASTN	827	1e-105	81
45	1	700092659H1	SATMON008	g1778820	BLASTN	1359	1e-104	92
46	1	LIB143-038-Q1-E1-H11	LIB143	g450548	BLASTN	1345	1e-103	84
47	1	LIB3062-028-Q1-K1-F12	LIB3062	g1778820	BLASTN	1082	1e-102	82
48	1	700103135H1	SATMON010	g1778820	BLASTN	1306	1e-100	93

49	1	LIB3078-050-Q1-K1-D9	LIB3078	g1778820	BLASTN	1309	1e-100	86
50	1	700084823H1	SATMON011	g1778820	BLASTN	1311	1e-100	90
51	1	700201311H1	SATMON003	g1778820	BLASTN	960	1e-97	89
52	1	700265914H1	SATMON017	g1778820	BLASTN	1275	1e-97	91
53	1	700205948H1	SATMON003	g1778820	BLASTN	983	1e-96	93
54	1	LIB189-003-Q1-E1-A6	LIB189	g450548	BLASTN	1262	1e-96	86
55	1	LIB143-017-Q1-E1-B9	LIB143	g1778820	BLASTN	779	1e-95	86
56	1	700097434H1	SATMON009	g450548	BLASTN	1247	1e-95	90
57	1	700089089H1	SATMON011	g450548	BLASTN	1251	1e-95	89
58	1	700071949H1	SATMON007	g1778820	BLASTN	1256	1e-95	87
59	1	700047892H1	SATMON003	g1778820	BLASTN	1236	1e-94	91
60	1	700077246H1	SATMON007	g450548	BLASTN	1237	1e-94	89
61	1	700085681H1	SATMON011	g1778820	BLASTN	1237	1e-94	89
62	1	LIB3067-010-Q1-K1-A7	LIB3067	g1778820	BLASTN	1244	1e-94	83
63	1	700619961H1	SATMON034	g450548	BLASTN	962	1e-93	89
64	1	700087395H1	SATMON011	g450548	BLASTN	1040	1e-93	89
65	1	700240431H1	SATMON010	g1778820	BLASTN	1156	1e-93	92
66	1	700053822H1	SATMON011	g1778820	BLASTN	1224	1e-93	91
67	1	700103856H1	SATMON010	g450548	BLASTN	1224	1e-93	88
68	1	700083703H1	SATMON011	g450548	BLASTN	1229	1e-93	89
69	1	700087360H1	SATMON011	g450548	BLASTN	1234	1e-93	90
70	1	700104713H1	SATMON010	g450548	BLASTN	1188	1e-92	87
71	1	700265996H1	SATMON017	g450548	BLASTN	1214	1e-92	88
72	1	700264870H1	SATMON017	g450548	BLASTN	945	1e-91	89
73	1	700219348H1	SATMON011	g1778820	BLASTN	1203	1e-91	91
74	1	700095527H1	SATMON008	g450548	BLASTN	1153	1e-90	88
75	1	LIB3068-023-Q1-K1-G5	LIB3068	g1778820	BLASTN	1187	1e-90	89
76	1	700102547H1	SATMON010	g1778820	BLASTN	1194	1e-90	88
77	1	700332179H1	SATMON019	g450548	BLASTN	1054	1e-89	88
78	1	700405470H1	SATMON029	g450548	BLASTN	1071	1e-89	90
79	1	LIB3069-043-Q1-K1-G2	LIB3069	g450548	BLASTN	1175	1e-89	84
80	1	700451332H1	SATMON028	g450548	BLASTN	1176	1e-89	90
81	1	700028108H1	SATMON003	g450548	BLASTN	1180	1e-89	89
82	1	700076154H1	SATMON007	g1778820	BLASTN	1180	1e-89	89
83	1	700089770H1	SATMON011	g450548	BLASTN	1185	1e-89	89
84	1	700051882H1	SATMON003	g1778820	BLASTN	844	1e-88	91
85	1	700242942H1	SATMON010	g1778820	BLASTN	1163	1e-88	91
86	1	700094996H1	SATMON008	g1778820	BLASTN	1166	1e-88	88
87	1	700476239H1	SATMON025	g450548	BLASTN	1166	1e-88	89
88	1	700219530H1	SATMON011	g1778820	BLASTN	1005	1e-87	91
89	1	700049726H1	SATMON003	g1778820	BLASTN	1041	1e-87	89
90	1	700096904H1	SATMON008	g1778820	BLASTN	1157	1e-87	86
91	1	700344026H1	SATMON021	g450548	BLASTN	1158	1e-87	88
92	1	700105590H1	SATMON010	g1778820	BLASTN	1139	1e-86	89
93	1	700465283H1	SATMON025	g450548	BLASTN	1145	1e-86	89
94	1	700104341H1	SATMON010	g450548				

103	I	700094764H1	SATMON008	g1778820	BLASTN	977	1e-84	86
104	I	700163030H1	SATMON013	g1778820	BLASTN	1011	1e-84	93
105	I	700466542H1	SATMON025	g450548	BLASTN	1085	1e-84	89
106	I	700047380H1	SATMON003	g450548	BLASTN	1096	1e-84	89
107	I	700456429H1	SATMON029	g450548	BLASTN	1118	1e-84	88
108	I	700096049H1	SATMON008	g1778820	BLASTN	1122	1e-84	86
109	I	700075906H1	SATMON007	g1778820	BLASTN	838	1e-83	86
110	I	LIB3067-048-Q1-K1-G3	LIB3067	g1778820	BLASTN	1045	1e-83	86
111	I	700214043H1	SATMON016	g450548	BLASTN	1103	1e-83	88
112	I	700096909H1	SATMON008	g1778820	BLASTN	1103	1e-83	90
113	I	701184635H1	SATMONN06	g1778820	BLASTN	1104	1e-83	87
114	I	700158969H1	SATMON012	g1778820	BLASTN	1107	1e-83	93
115	I	700475902H1	SATMON025	g450548	BLASTN	1112	1e-83	89
116	I	700207076H1	SATMON003	g1778820	BLASTN	1113	1e-83	90
117	I	700161901H1	SATMON012	g1778820	BLASTN	1091	1e-82	91
118	I	700452183H1	SATMON028	g450548	BLASTN	1095	1e-82	88
119	I	700452326H1	SATMON028	g450548	BLASTN	1098	1e-82	88
120	I	700106918H1	SATMON010	g450548	BLASTN	1101	1e-82	88
121	I	700220146H1	SATMON011	g450548	BLASTN	1083	1e-81	89
122	I	700221060H1	SATMON011	g450548	BLASTN	1084	1e-81	88
123	I	700243344H1	SATMON010	g1778820	BLASTN	1084	1e-81	92
124	I	700475790H1	SATMON025	g450548	BLASTN	1084	1e-81	89
125	I	700452691H1	SATMON028	g1778820	BLASTN	1086	1e-81	87
126	I	700243733H1	SATMON010	g450548	BLASTN	1086	1e-81	90
127	I	LIB3059-018-Q1-K1-D10	LIB3059	g1778820	BLASTN	830	1e-80	91
128	I	700165958H1	SATMON013	g450548	BLASTN	1070	1e-80	89
129	I	700082659H1	SATMON011	g1778820	BLASTN	1071	1e-80	88
130	I	700172557H1	SATMON013	g1778820	BLASTN	1074	1e-80	92
131	I	700221526H1	SATMON011	g450548	BLASTN	1075	1e-80	88
132	I	700456696H1	SATMON029	g450548	BLASTN	1077	1e-80	89
133	I	700430776H1	SATMONN01	g1778820	BLASTN	574	1e-79	90
134	I	LIB3062-025-Q1-K1-B11	LIB3062	g450548	BLASTN	585	1e-79	85
135	I	700077126H1	SATMON007	g1778820	BLASTN	743	1e-79	88
136	I	700160310H1	SATMON012	g1778820	BLASTN	752	1e-79	91
137	I	700030466H1	SATMON003	g1778820	BLASTN	757	1e-79	83
138	I	700210204H1	SATMON016	g1778820	BLASTN	844	1e-79	91
139	I	700094716H1	SATMON008	g1778820	BLASTN	857	1e-79	86
140	I	700157051H1	SATMON012	g1778820	BLASTN	937	1e-79	89
141	I	700241415H1	SATMON010	g450548	BLASTN	1055	1e-79	90
142	I	700160127H1	SATMON012	g450548	BLASTN	1056	1e-79	88
143	I	700205485H1	SATMON003	g1778820	BLASTN	1059	1e-79	90
144	I	700475930H1	SATMON025	g450548	BLASTN	1060	1e-79	89
145	I	700161902H1	SATMON012	g1778820	BLASTN	1060	1e-79	91
146	I	700213573H1	SATMON016	g450548	BLASTN	1061	1e-79	89
147	I	700207677H1	SATMON016	g450548	BLASTN	1061	1e-79	88
148	I	700581117H1	SATMON031	g450548	BLASTN	1065	1e-79	88
149	I	700451418H1	SATMON028	g450548	BLASTN	546	1e-78	89
150	I	700212606H1	SATMON016	g1778820	BLASTN	1044	1e-78	85
151	I	700222605H1	SATMON011	g1778820	BLASTN	1047	1e-78	86
152	I	700050542H1	SATMON003	g1778820	BLASTN	1053	1e-78	86
153	I	700450709H1	SATMON028	g450548	BLASTN	834	1e-77	88
154	I	700213368H1	SATMON016	g450548	BLASTN	861	1e-77	89
155	I	700095926H1	SATMON008	g1778820	BLASTN	1030	1e-77	86
156	I	700261686H1	SATMON017	g1778820	BLASTN	1034	1e-77	89



157	1	700235993H1	SATMON010	g450548	BLASTN	1037	1e-77	88
158	1	700466152H1	SATMON025	g450548	BLASTN	1038	1e-77	89
159	1	700266410H1	SATMON017	g1778820	BLASTN	1041	1e-77	85
160	1	700216991H1	SATMON016	g1778820	BLASTN	491	1e-76	92
161	1	700464957H1	SATMON025	g450548	BLASTN	607	1e-76	88
162	1	700160463H1	SATMON012	g1778820	BLASTN	1018	1e-76	91
163	1	700158224H1	SATMON012	g1778820	BLASTN	1018	1e-76	92
164	1	700469971H1	SATMON025	g1778820	BLASTN	1020	1e-76	86
165	1	700222731H1	SATMON011	g450548	BLASTN	1022	1e-76	88
166	1	700087434H1	SATMON011	g1778820	BLASTN	1023	1e-76	86
167	1	700094482H1	SATMON008	g1778820	BLASTN	1023	1e-76	86
168	1	700235976H1	SATMON010	g450548	BLASTN	1028	1e-76	89
169	1	700088266H1	SATMON011	g1778820	BLASTN	983	1e-75	92
170	1	700075882H1	SATMON007	g1778820	BLASTN	1009	1e-75	83
171	1	700243324H1	SATMON010	g1778820	BLASTN	1012	1e-75	86
172	1	700159625H2	SATMON012	g450548	BLASTN	1015	1e-75	90
173	1	700204422H1	SATMON003	g1778820	BLASTN	1015	1e-75	88
174	1	700048228H1	SATMON003	g1778820	BLASTN	741	1e-74	85
175	1	700072474H1	SATMON007	g1778820	BLASTN	799	1e-74	85
176	1	700477790H1	SATMON025	g450548	BLASTN	960	1e-74	89
177	1	LIB3059-022-Q1-K1-A9	LIB3059	g1778820	BLASTN	995	1e-74	86
178	1	700241633H1	SATMON010	g450548	BLASTN	997	1e-74	89
179	1	700093978H1	SATMON008	g1778820	BLASTN	999	1e-74	89
180	1	700238569H1	SATMON010	g450548	BLASTN	844	1e-73	88
181	1	700455160H1	SATMON029	g450548	BLASTN	849	1e-73	86
182	1	700262745H1	SATMON017	g1778820	BLASTN	984	1e-73	85
183	1	700405029H1	SATMON027	g1778820	BLASTN	990	1e-73	90
184	1	700195230H1	SATMON014	g450548	BLASTN	991	1e-73	88
185	1	700264753H1	SATMON017	g1778820	BLASTN	993	1e-73	90
186	1	700050142H1	SATMON003	g1778820	BLASTN	433	1e-72	86
187	1	700452677H1	SATMON028	g450548	BLASTN	919	1e-72	87
188	1	700168870H1	SATMON013	g1778820	BLASTN	971	1e-72	90
189	1	700023193H1	SATMON003	g450548	BLASTN	972	1e-72	88
190	1	700086582H1	SATMON011	g1778820	BLASTN	980	1e-72	84
191	1	700457710H1	SATMON029	g450548	BLASTN	982	1e-72	87
192	1	700477601H1	SATMON025	g450548	BLASTN	635	1e-71	88
193	1	700377730H1	SATMON019	g1778820	BLASTN	691	1e-71	87
194	1	700169782H1	SATMON013	g1778820	BLASTN	960	1e-71	91
195	1	700461113H1	SATMON033	g1778820	BLASTN	962	1e-71	85
196	1	700258669H1	SATMON017	g1778820	BLASTN	963	1e-71	90
197	1	700454253H1	SATMON029	g1778820	BLASTN	964	1e-71	85
198	1	700092376H1	SATMON008	g1778820	BLASTN	780	1e-70	83
199	1	700242885H1	SATMON010	g960356	BLASTN	946	1e-70	86
200	1	700094333H1	SATMON008	g1778820	BLASTN	947	1e-70	86
201	1	700575124H1	SATMON030	g450548	BLASTN	948	1e-70	82
202	1	700072544H1	SATMON007					

211	1	700424156H1	SATMONN01	g450548	BLASTN	752	1e-68	85
212	1	700405486H1	SATMON029	g450548	BLASTN	864	1e-68	91
213	1	LIB189-026-Q1-E1-H12	LIB189	g1778820	BLASTN	879	1e-68	87
214	1	700106172H1	SATMON010	g1778820	BLASTN	925	1e-68	85
215	1	700096890H1	SATMON008	g1778820	BLASTN	926	1e-68	90
216	1	700154404H1	SATMON007	g450548	BLASTN	928	1e-68	89
217	1	700468337H1	SATMON025	g450548	BLASTN	445	1e-67	88
218	1	700158827H1	SATMON012	g1778820	BLASTN	522	1e-67	87
219	1	700241744H1	SATMON010	g1778820	BLASTN	575	1e-67	85
220	1	700624633H1	SATMON034	g960356	BLASTN	644	1e-67	85
221	1	700154564H1	SATMON007	g1778820	BLASTN	735	1e-67	85
222	1	700172424H1	SATMON013	g450548	BLASTN	912	1e-67	90
223	1	700096287H1	SATMON008	g1778820	BLASTN	914	1e-67	86
224	1	700207638H1	SATMON016	g1778820	BLASTN	914	1e-67	86
225	1	700093735H1	SATMON008	g960356	BLASTN	920	1e-67	89
226	1	700159494H1	SATMON012	g1778820	BLASTN	899	1e-66	89
227	1	700236013H1	SATMON010	g1778820	BLASTN	900	1e-66	83
228	1	700220267H1	SATMON011	g1778820	BLASTN	439	1e-65	84
229	1	700477578H1	SATMON025	g450548	BLASTN	576	1e-65	90
230	1	700047743H1	SATMON003	g1778820	BLASTN	601	1e-65	82
231	1	700343041H1	SATMON021	g1778820	BLASTN	633	1e-65	86
232	1	700159886H1	SATMON012	g450548	BLASTN	803	1e-65	85
233	1	700570242H1	SATMON030	g450548	BLASTN	834	1e-65	85
234	1	700021930H1	SATMON001	g1778820	BLASTN	888	1e-65	87
235	1	700454959H1	SATMON029	g450548	BLASTN	890	1e-65	89
236	1	700171336H1	SATMON013	g1778820	BLASTN	890	1e-65	89
237	1	700105361H1	SATMON010	g1778820	BLASTN	806	1e-64	87
238	1	LIB3068-031-Q1-K1-B1	LIB3068	g450548	BLASTN	853	1e-64	89
239	1	700236324H1	SATMON010	g450548	BLASTN	875	1e-64	89
240	1	700150620H1	SATMON007	g450548	BLASTN	880	1e-64	87
241	1	700220648H1	SATMON011	g450548	BLASTN	881	1e-64	90
242	1	700150733H1	SATMON007	g450548	BLASTN	881	1e-64	85
243	1	700157367H1	SATMON012	g1778820	BLASTN	882	1e-64	85
244	1	700259676H1	SATMON017	g1778820	BLASTN	885	1e-64	88
245	1	700616490H1	SATMON033	g450548	BLASTN	886	1e-64	82
246	1	700102511H1	SATMON010	g450548	BLASTN	695	1e-63	89
247	1	700202805H1	SATMON003	g1778820	BLASTN	817	1e-63	92
248	1	700105685H1	SATMON010	g1778820	BLASTN	866	1e-63	84
249	1	700106113H1	SATMON010	g450548	BLASTN	873	1e-63	91
250	1	700444778H1	SATMON027	g1778820	BLASTN	343	1e-62	84
251	1	700103584H1	SATMON010	g450548	BLASTN	521	1e-62	86
252	1	LIB189-008-Q1-E1-D9	LIB189	g1778820	BLASTN	850	1e-62	91
253	1	700155684H1	SATMON007	g1778820	BLASTN	852	1e-62	85
254	1	700261639H1	SATMON017	g1778820	BLASTN	853	1e-62	89
255	1	700158367H1	SATMON012	g450548	BLASTN	856	1e-62	81
256	1	700153242H1	SATMON007	g1778820	BLASTN	859	1e-62	90
257	1	700210738H1	SATMON016	g1778820	BLASTN	859	1e-62	90
258	1	700203008H1	SATMON003	g450548	BLASTN	698	1e-61	86
259	1	700206081H1	SATMON003	g1778820	BLASTN	840	1e-61	92
260	1	700028643H1	SATMON003	g1778820	BLASTN	846	1e-61	85
261	1	700223914H1	SATMON011	g1778820	BLASTN	849	1e-61	84
262	1	700571455H1	SATMON030	g1778820	BLASTN	378	1e-60	88
263	1	700075374H1	SATMON007	g1778820	BLASTN	830	1e-60	85
264	1	700150452H1	SATMON007	g450548	BLASTN	831	1e-60	89

265	1	700261318H1	SATMON017	g1778820	BLASTN	831	1e-60	83
266	1	700616390H1	SATMON033	g450548	BLASTN	835	1e-60	92
267	1	700208718H1	SATMON016	g450548	BLASTN	561	1e-59	89
268	1	700448948H1	SATMON028	g1778820	BLASTN	653	1e-59	83
269	1	LIB3060-035-Q1-K1-H3	LIB3060	g450548	BLASTN	734	1e-59	85
270	1	700049753H1	SATMON003	g450548	BLASTN	769	1e-59	90
271	1	700154489H1	SATMON007	g1778820	BLASTN	814	1e-59	83
272	1	700170783H1	SATMON013	g1778820	BLASTN	816	1e-59	87
273	1	700237571H1	SATMON010	g450548	BLASTN	817	1e-59	89
274	1	700154872H1	SATMON007	g1778820	BLASTN	822	1e-59	85
275	1	700025620H1	SATMON004	g1778820	BLASTN	686	1e-58	84
276	1	700158255H1	SATMON012	g450548	BLASTN	803	1e-58	86
277	1	700159282H1	SATMON012	g450548	BLASTN	805	1e-58	83
278	1	700222171H1	SATMON011	g1778820	BLASTN	807	1e-58	89
279	1	700205003H1	SATMON003	g1778820	BLASTN	441	1e-57	86
280	1	700049209H1	SATMON003	g1778820	BLASTN	443	1e-57	90
281	1	700016430H1	SATMON001	g1778820	BLASTN	492	1e-57	86
282	1	700212936H1	SATMON016	g450548	BLASTN	564	1e-57	86
283	1	700156939H1	SATMON012	g1778820	BLASTN	801	1e-57	84
284	1	700222183H1	SATMON011	g1778820	BLASTN	561	1e-56	83
285	1	700203453H1	SATMON003	g1778820	BLASTN	784	1e-56	90
286	1	700167708H1	SATMON013	g1778820	BLASTN	560	1e-55	83
287	1	700214356H1	SATMON016	g1778820	BLASTN	693	1e-55	87
288	1	700475377H1	SATMON025	g450548	BLASTN	705	1e-55	90
289	1	700029625H1	SATMON003	g450548	BLASTN	712	1e-55	88
290	1	700202091H1	SATMON003	g1778820	BLASTN	774	1e-55	90
291	1	700264594H1	SATMON017	g450548	BLASTN	775	1e-55	89
292	1	700074969H1	SATMON007	g450548	BLASTN	777	1e-55	87
293	1	LIB3068-005-Q1-K1-A9	LIB3068	g1778820	BLASTN	389	1e-54	80
294	1	700207147H1	SATMON017	g1778820	BLASTN	537	1e-54	88
295	1	LIB189-004-Q1-E1-F11	LIB189	g960356	BLASTN	757	1e-54	87
296	1	700171222H1	SATMON013	g1778820	BLASTN	757	1e-54	93
297	1	700239903H1	SATMON010	g1778820	BLASTN	760	1e-54	84
298	1	700048208H1	SATMON003	g1778820	BLASTN	762	1e-54	90
299	1	700264085H1	SATMON017	g450548	BLASTN	764	1e-54	87
300	1	700155761H1	SATMON007	g1778820	BLASTN	579	1e-53	85
301	1	700216516H1	SATMON016	g450548	BLASTN	380	1e-52	89
302	1	LIB3068-044-Q1-K1-F12	LIB3068	g450548	BLASTN	492	1e-52	73
303	1	700344420H1	SATMON021	g450548	BLASTN	534	1e-52	79
304	1	700219767H1	SATMON011	g450548	BLASTN	732	1e-52	88
305	1	700153757H1	SATMON007	g1778820	BLASTN	737	1e-52	89
306	1	700165841H1	SATMON013	g450548	BLASTN	738	1e-52	90
307	1	700381966H1	SATMON023	g1778820	BLASTN	740	1e-52	86
308	1	700170427H1	SATMON013	g450548	BLASTN	687	1e-51	89
309	1	700094344H1	SATMON008	g1778820	BLASTN	725	1e-51	93
310	1	700052248H1	SATMON003	g1778820	BLASTN	726	1e-51	84
311	1	700050628H1	SATMON003	g450548	BLASTN	726	1e-51	89
312	1	700223031H1	SATMON011	g1778820	BLASTN	729	1e-51	89
313	1	700475686H1	SATMON025	g450548	BLASTN	660	1e-50	89
314	1	700170325H1	SATMON013	g2305013	BLASTN	709	1e-50	82
315	1	700221107H1	SATMON011	g450548	BLASTN	698	1e-49	89
316	1	700612589H1	SATMON033	g960356	BLASTN	703	1e-49	89
317	1	700153770H1	SATMON007	g1778820	BLASTN	705	1e-49	88
318	1	LIB3078-006-Q1-K1-E7	LIB3078	g1778820	BLASTN	705	1e-49	79

319	1	700239724H1	SATMON010	g1778820	BLASTN	507	1e-48	78
320	1	700356777H1	SATMON024	g1778820	BLASTN	683	1e-48	86
321	1	700241568H1	SATMON010	g960356	BLASTN	691	1e-48	88
322	1	700051294H1	SATMON003	g1778820	BLASTN	462	1e-47	88
323	1	700343661H1	SATMON021	g450548	BLASTN	507	1e-47	81
324	1	700029419H1	SATMON003	g1778820	BLASTN	547	1e-47	87
325	1	700165936H1	SATMON013	g1778820	BLASTN	671	1e-47	83
326	1	700026152H1	SATMON003	g960356	BLASTN	673	1e-47	89
327	1	700075671H1	SATMON007	g450548	BLASTN	429	1e-46	89
328	1	700156674H1	SATMON012	g1778820	BLASTN	659	1e-46	91
329	1	700094981H1	SATMON008	g1778820	BLASTN	662	1e-46	85
330	1	700092879H1	SATMON008	g1778820	BLASTN	668	1e-46	87
331	1	700240685H1	SATMON010	g1778820	BLASTN	484	1e-45	84
332	1	700150286H1	SATMON007	g1778820	BLASTN	647	1e-45	82
333	1	700104990H1	SATMON010	g450548	BLASTN	652	1e-45	89
334	1	700203829H1	SATMON003	g450548	BLASTN	652	1e-45	87
335	1	700153718H1	SATMON007	g167961	BLASTN	656	1e-45	91
336	1	700050841H1	SATMON003	g450548	BLASTN	571	1e-44	86
337	1	700268037H1	SATMON017	g2305013	BLASTN	636	1e-44	86
338	1	700153630H1	SATMON007	g1778820	BLASTN	637	1e-44	82
339	1	700475317H1	SATMON025	g450548	BLASTN	530	1e-43	87
340	1	701163127H1	SATMONN04	g960356	BLASTN	626	1e-43	88
341	1	700203688H1	SATMON003	g960356	BLASTN	627	1e-43	89
342	1	700049893H1	SATMON003	g1778820	BLASTN	506	1e-42	90
343	1	700449155H1	SATMON028	g1778820	BLASTN	443	1e-41	85
344	1	701183780H1	SATMONN06	g450548	BLASTN	558	1e-41	86
345	1	700242162H1	SATMON010	g1778820	BLASTN	600	1e-41	90
346	1	700466994H1	SATMON025	g450548	BLASTN	604	1e-41	91
347	1	700259823H1	SATMON017	g450548	BLASTN	607	1e-41	83
348	1	700346242H1	SATMON021	g450548	BLASTN	608	1e-41	86
349	1	700156395H1	SATMON007	g1778820	BLASTN	609	1e-41	90
350	1	700236835H1	SATMON010	g1778820	BLASTN	335	1e-40	81
351	1	700172385H1	SATMON013	g1778820	BLASTN	397	1e-40	87
352	1	700210466H1	SATMON016	g1778820	BLASTN	586	1e-40	81
353	1	700257303H1	SATMON017	g1778820	BLASTN	589	1e-40	81
354	1	LIB3067-027-Q1-K1-G1	LIB3067	g1778820	BLASTN	623	1e-40	87
355	1	LIB3066-025-Q1-K1-D1	LIB3066	g450548	BLASTN	524	1e-39	85
356	1	700106853H1	SATMON010	g450548	BLASTN	580	1e-39	86
357	1	700160540H1	SATMON012	g960356	BLASTN	581	1e-39	88
358	1	700157780H1	SATMON012	g960356	BLASTN	581	1e-39	88
359	1	700149801H1	SATMON007	g450548	BLASTN	565	1e-38	86
360	1	700353243H1	SATMON024	g450548	BLASTN	570	1e-38	86
361	1	700166171H1	SATMON013	g1778820	BLASTN	254	1e-37	79
362	1	700142509H1	SATMON012	g960356	BLASTN	556	1e-37	88
363	1	700242131H1	SATMON010	g450548	BLASTN	560	1e-37	85
364	1	700455643H1	SATMON029	g450548	BLASTN	539	1e-36	86
365	1	700150248H1	SATMON007	g1778820	BLASTN	547	1e-36	87
366	1	700208549H1	SATMON016	g450548	BLASTN	559	1e-36	88
367	1	700027193H1	SATMON003	g450548	BLASTN	529	1e-35	86
368	1	700221390H1	SATMON011	g450548	BLASTN	530	1e-35	85
369	1	700455647H1	SATMON029	g450548	BLASTN	531	1e-35	85
370	1	700260103H1	SATMON017	g1778820	BLASTN	531	1e-35	80
371	1	700167344H1	SATMON013	g1778820	BLASTN	536	1e-35	88
372	1	700089913H1	SATMON011	g960356	BLASTN	546	1e-35	88

373	1	700570573H1	SATMON030	g450548	BLASTN	300	1e-34	89
374	1	700169889H1	SATMON013	g1778820	BLASTN	519	1e-34	89
375	1	700262857H1	SATMON017	g1778820	BLASTN	521	1e-34	87
376	1	700142644H2	SATMON013	g1778820	BLASTN	522	1e-34	87
377	1	700224417H1	SATMON011	g450548	BLASTN	522	1e-34	91
378	1	700073882H1	SATMON007	g450548	BLASTN	531	1e-34	87
379	1	700085803H1	SATMON011	g450548	BLASTN	338	1e-33	89
380	1	700162323H1	SATMON012	g1778820	BLASTN	513	1e-33	86
381	1	700468306H1	SATMON025	g450548	BLASTN	519	1e-33	89
382	1	700443224H2	SATMON026	g450548	BLASTN	275	1e-32	83
383	1	LIB3066-054-Q1-K1-E3	LIB3066	g450548	BLASTN	495	1e-32	87
384	1	700211827H1	SATMON016	g1778820	BLASTN	489	1e-31	80
385	1	700048741H1	SATMON003	g450548	BLASTN	500	1e-31	88
386	1	700613620H1	SATMON033	g1778820	BLASTN	385	1e-30	88
387	1	700029203H1	SATMON003	g1778820	BLASTN	461	1e-29	84
388	1	700378431H1	SATMON020	g450548	BLASTN	466	1e-29	89
389	1	700455641H1	SATMON029	g450548	BLASTN	472	1e-29	85
390	1	700447867H1	SATMON027	g960356	BLASTN	473	1e-29	88
391	1	700447511H1	SATMON027	g450548	BLASTN	474	1e-29	88
392	1	700025851H1	SATMON003	g450548	BLASTN	479	1e-29	88
393	1	LIB3066-024-Q1-K1-H4	LIB3066	g1778820	BLASTN	481	1e-29	81
394	1	LIB143-025-Q1-E1-C4	LIB143	g450549	BLASTX	64	1e-28	70
395	1	700242282H1	SATMON010	g1778820	BLASTN	280	1e-28	85
396	1	700087244H1	SATMON011	g450548	BLASTN	464	1e-28	88
397	1	700458127H1	SATMON029	g450548	BLASTN	238	1e-27	82
398	1	700025767H1	SATMON003	g1778820	BLASTN	438	1e-26	86
399	1	700235401H1	SATMON010	g450548	BLASTN	442	1e-26	88
400	1	700029457H1	SATMON003	g450548	BLASTN	446	1e-26	89
401	1	700266174H1	SATMON017	g450548	BLASTN	447	1e-26	89
402	1	700349254H1	SATMON023	g1778820	BLASTN	315	1e-25	85
403	1	LIB3068-041-Q1-K1-H6	LIB3068	g450548	BLASTN	438	1e-25	86
404	1	700092889H1	SATMON008	g1778820	BLASTN	397	1e-24	92
405	1	700049044H1	SATMON003	g1778820	BLASTN	425	1e-24	90
406	1	700202710H1	SATMON003	g960357	BLASTX	114	1e-19	97
407	1	700155117H1	SATMON007	g450548	BLASTN	314	1e-19	90
408	1	700449619H1	SATMON028	g1778820	BLASTN	341	1e-19	89
409	1	700150336H1	SATMON007	g1778820	BLASTN	343	1e-19	87
410	1	700166223H1	SATMON013	g960357	BLASTX	181	1e-18	97
411	1	700153796H1	SATMON007	g2305013	BLASTN	300	1e-16	82
412	1	700053266H1	SATMON008	g450548	BLASTN	292	1e-15	83
413	1	700405227H1	SATMON028	g450548	BLASTN	313	1e-15	90
414	1	700397410H1	SATMONN01	g17262	BLASTX	147	1e-14	96
415	1	700211524H1	SATMON016	g1033190	BLASTX	153	1e-14	100
416	1	700281415H2	SATMON019	g2315140	BLASTX	139	1e-13	89
417	1	700429873H1	SATMONN01	g16961	BLASTX	133	1e-12	94
418	1	700239660H1	SATMON010	g450548	BLASTN	245	1e-12	86
419	1	700213596H1	SATMON016	g450548	BLASTN	258	1e-12	95
420	1	700152367H1	SATMON007	g450548	BLASTN	273	1e-12	88
421	1	700452014H1	SATMON028	g17262	BLASTX	76	1e-11	72
422	1	700357106H1	SATMON024	g1724104	BLASTX	132	1e-11	100
423	1	700468611H1	SATMON025	g450549	BLASTX	93	1e-10	93
424	1	700213526H1	SATMON016	g450549	BLASTX	127	1e-10	96
425	1	700267065H1	SATMON017	g450549	BLASTX	130	1e-10	96
426	1	700152044H1	SATMON007	g450549	BLASTX	88	1e-8	93

427	1	700159090H1	SATMON012	g169665	BLASTX	113	1e-8	81
428	1	700266734H1	SATMON017	g450549	BLASTX	119	1e-8	96
429	1	700405367H1	SATMON029	g1778820	BLASTN	231	1e-8	65
1635	-700555532	700555532H1	SOYMON001	g429103	BLASTN	920	1e-67	84
1636	-700649594	700649594H1	SOYMON003	g609559	BLASTX	186	1e-23	85
1637	-700750590	700750590H1	SOYMON014	g609224	BLASTN	363	1e-40	79
1638	-700755802	700755802H1	SOYMON014	g609224	BLASTN	479	1e-43	74
1639	-700869211	700869211H1	SOYMON016	g169665	BLASTX	146	1e-13	92
1640	-700891960	700891960H1	SOYMON024	g726031	BLASTN	589	1e-56	82
1641	-700900377	700900377H1	SOYMON027	g1655577	BLASTN	235	1e-8	78
1642	-700902427	700902427H1	SOYMON027	g1655576	BLASTX	151	1e-13	76
1643	-700941686	700941686H1	SOYMON024	g497899	BLASTN	442	1e-26	89
1644	-700952418	700952418H1	SOYMON022	g726030	BLASTX	146	1e-17	83
1645	-700979651	700979651H2	SOYMON009	g1655579	BLASTN	1006	1e-75	84
1646	-700982809	700982809H1	SOYMON009	g1655579	BLASTN	945	1e-69	82
1647	-700982867	700982867H1	SOYMON009	g1127582	BLASTN	868	1e-63	78
1648	-701056884	701056884H1	SOYMON032	g609556	BLASTN	726	1e-51	84
1649	-701117318	701117318H1	SOYMON037	g609224	BLASTN	451	1e-37	80
1650	-701118224	701118224H1	SOYMON037	g2305013	BLASTN	285	1e-19	72
1651	-701121264	701121264H1	SOYMON037	g166873	BLASTN	238	1e-8	82
1652	-701122908	701122908H1	SOYMON037	g16508	BLASTN	444	1e-26	83
1653	-701128589	701128589H1	SOYMON037	g16508	BLASTN	539	1e-36	90
1654	-GM12798	LIB3049-039-Q1-E1-F2	LIB3049	g16508	BLASTN	578	1e-37	73
1655	-GM14331	LIB3049-055-Q1-E1-F5	LIB3049	g167961	BLASTN	497	1e-30	62
1656	-GM30881	LIB3050-005-Q1-K1-G1	LIB3050	g1655577	BLASTN	543	1e-34	82
1657	-GM30911	LIB3050-005-Q1-K1-B8	LIB3050	g1655577	BLASTN	387	1e-26	71
1658	-GM33921	LIB3051-028-Q1-K1-A9	LIB3051	g16508	BLASTN	338	1e-32	73
1659	12644	701131794H1	SOYMON038	g429107	BLASTN	877	1e-64	84
1660	12644	701142515H1	SOYMON038	g429107	BLASTN	871	1e-63	84
1661	12644	700888494H1	SOYMON024	g429107	BLASTN	662	1e-46	83
1662	16	LIB3030-009-Q1-B1-C1	LIB3030	g429105	BLASTN	1439	1e-119	84
1663	16	LIB3051-106-Q1-K1-B5	LIB3051	g609224	BLASTN	1516	1e-117	86
1664	16	LIB3050-023-Q1-K1-A12	LIB3050	g1724103	BLASTN	1388	1e-106	83
1665	16	LIB3028-003-Q1-B1-G11	LIB3028	g609224	BLASTN	1313	1e-100	87
1666	16	LIB3054-010-Q1-N1-E2	LIB3054	g609224	BLASTN	920	1e-95	87
1667	16	700651294H1	SOYMON003	g609224	BLASTN	672	1e-94	86
1668	16	LIB3027-007-Q1-B1-G3	LIB3027	g16508	BLASTN	891	1e-93	83
1669	16	LIB3053-013-Q1-N1-H9	LIB3053	g609224	BLASTN	996	1e-93	87
1670	16	LIB3051-061-Q1-K1-C7	LIB3051	g16508	BLASTN	1195	1e-93	86
1671	16	LIB3065-005-Q1-N1-A6	LIB3065	g609224	BLASTN	707	1e-91	78
1672	16	LIB3050-008-Q1-E1-E3	LIB3050	g609224	BLASTN	1102	1e-87	87
1673	16	LIB3051-011-Q1-E1-B2	LIB3051	g16508	BLASTN	1153	1e-87	82
1674	16	LIB3030-010-Q1-B1-F8	LIB3030	g609224	BLASTN	988	1e-86	83
1675	16	700652104H1	SOYMON003	g1655577	BLASTN	990	1e-86	83
1676	16	701205279H1	SOYMON035	g609556	BLASTN	1125	1e-85	86
1677	16	700662183H1	SOYMON005	g609224	BLASTN	678	1e-83	84
1678	16	LIB3051-039-Q1-K1-F5	LIB3051	g169664	BLASTN	1104	1e-83	86
1679	16	700557616H1	SOYMON001	g609556	BLASTN	1111	1e-83	86
1680	16	LIB3030-002-Q1-B1-C6	LIB3030	g16508	BLASTN	1113	1e-83	83
1681	16	700865235H1	SOYMON016	g1724103	BLASTN	1090	1e-82	84
1682	16	700563340H1	SOYMON002	g609224	BLASTN	911	1e-80	86
1683	16	LIB3040-044-Q1-E1-D7	LIB3040	g166873	BLASTN	1052	1e-80	82
1684	16	LIB3040-060-Q1-E1-D9	LIB3040	g609224	BLASTN	1071	1e-80	84
1685	16	LIB3049-001-Q1-E1-F12	LIB3049	g16508	BLASTN	1074	1e-80	84

1686	16	LIB3028-006-Q1-B1-F1	LIB3028	g16508	BLASTN	857	1e-79	83
1687	16	LIB3049-033-Q1-E1-G5	LIB3049	g2315139	BLASTN	933	1e-79	81
1688	16	700978240H1	SOYMON009	g609224	BLASTN	984	1e-79	88
1689	16	700980802H1	SOYMON009	g862999	BLASTN	1060	1e-79	85
1690	16	700755908H1	SOYMON014	g1724103	BLASTN	1062	1e-79	88
1691	16	700562226H1	SOYMON002	g1724103	BLASTN	1065	1e-79	84
1692	16	701119101H1	SOYMON037	g609224	BLASTN	1048	1e-78	86
1693	16	700646291H1	SOYMON012	g1655577	BLASTN	1049	1e-78	86
1694	16	LIB3050-022-Q1-K1-B9	LIB3050	g16508	BLASTN	1049	1e-78	83
1695	16	LIB3028-030-Q1-B1-F8	LIB3028	g16508	BLASTN	1053	1e-78	83
1696	16	701143128H1	SOYMON038	g609556	BLASTN	737	1e-77	86
1697	16	LIB3040-041-Q1-E1-D10	LIB3040	g166873	BLASTN	861	1e-77	81
1698	16	700756363H1	SOYMON014	g609556	BLASTN	1031	1e-77	88
1699	16	700729963H1	SOYMON009	g1724103	BLASTN	1036	1e-77	88
1700	16	701063046H1	SOYMON033	g609224	BLASTN	1037	1e-77	86
1701	16	LIB3030-005-Q1-B1-G2	LIB3030	g609224	BLASTN	1038	1e-77	84
1702	16	700564331H1	SOYMON002	g609556	BLASTN	1039	1e-77	84
1703	16	700562958H1	SOYMON002	g1724103	BLASTN	1040	1e-77	88
1704	16	LIB3039-014-Q1-E1-F7	LIB3039	g16508	BLASTN	569	1e-76	84
1705	16	700753632H1	SOYMON014	g497899	BLASTN	668	1e-76	83
1706	16	LIB3049-048-Q1-E1-F2	LIB3049	g16508	BLASTN	722	1e-76	83
1707	16	700648911H1	SOYMON003	g1655577	BLASTN	983	1e-76	83
1708	16	LIB3040-027-Q1-E1-H3	LIB3040	g166873	BLASTN	1003	1e-76	81
1709	16	700664905H1	SOYMON005	g609556	BLASTN	1020	1e-76	86
1710	16	701133404H1	SOYMON038	g1724103	BLASTN	1023	1e-76	85
1711	16	701208780H1	SOYMON035	g1655577	BLASTN	1025	1e-76	85
1712	16	700902279H1	SOYMON027	g169664	BLASTN	1026	1e-76	88
1713	16	LIB3040-048-Q1-E1-G10	LIB3040	g16508	BLASTN	1030	1e-76	83
1714	16	LIB3040-028-Q1-E1-A4	LIB3040	g16508	BLASTN	979	1e-75	84
1715	16	700807586H1	SOYMON016	g609224	BLASTN	1006	1e-75	86
1716	16	700755486H1	SOYMON014	g609224	BLASTN	1006	1e-75	87
1717	16	700724920H1	SOYMON009	g609224	BLASTN	1009	1e-75	87
1718	16	701007494H2	SOYMON019	g1724103	BLASTN	1013	1e-75	85
1719	16	700568310H1	SOYMON002	g497899	BLASTN	834	1e-74	83
1720	16	701208819H1	SOYMON035	g609224	BLASTN	857	1e-74	87
1721	16	700985084H1	SOYMON009	g609224	BLASTN	872	1e-74	85
1722	16	701013074H1	SOYMON019	g726031	BLASTN	894	1e-74	88
1723	16	700650843H1	SOYMON003	g609224	BLASTN	924	1e-74	86
1724	16	700646037H1	SOYMON011	g609556	BLASTN	930	1e-74	84
1725	16	LIB3040-039-Q1-E1-H9	LIB3040	g166873	BLASTN	973	1e-74	80
1726	16	700847231H1	SOYMON021	g726031	BLASTN	999	1e-74	85
1727	16	700792293H1	SOYMON011	g609224	BLASTN	999	1e-74	86
1728	16	701109644H1	SOYMON036	g609556	BLASTN	1004	1e-74	86
1729	16	701122929H1	SOYMON037	g1724103	BLASTN	459		

1740	16	700868625H1	SOYMON016	g1724103	BLASTN	973	1e-72	85
1741	16	700992344H1	SOYMON011	g726031	BLASTN	973	1e-72	85
1742	16	701012719H1	SOYMON019	g1724103	BLASTN	973	1e-72	85
1743	16	700676769H1	SOYMON007	g609224	BLASTN	973	1e-72	87
1744	16	700946235H1	SOYMON024	g609224	BLASTN	975	1e-72	85
1745	16	700891218H1	SOYMON024	g1724103	BLASTN	975	1e-72	84
1746	16	700724907H1	SOYMON009	g609556	BLASTN	977	1e-72	84
1747	16	700833549H1	SOYMON019	g1655577	BLASTN	979	1e-72	85
1748	16	700942105H1	SOYMON024	g726031	BLASTN	980	1e-72	84
1749	16	700564290H1	SOYMON002	g726031	BLASTN	530	1e-71	84
1750	16	700891233H1	SOYMON024	g1724103	BLASTN	746	1e-71	86
1751	16	LIB3028-025-Q1-B1-B2	LIB3028	g609224	BLASTN	870	1e-71	85
1752	16	701120896H1	SOYMON037	g429105	BLASTN	959	1e-71	82
1753	16	LIB3051-027-Q1-K1-A9	LIB3051	g609224	BLASTN	962	1e-71	83
1754	16	701050696H1	SOYMON032	g609556	BLASTN	965	1e-71	86
1755	16	700653619H1	SOYMON003	g609224	BLASTN	966	1e-71	86
1756	16	701047994H1	SOYMON032	g1724103	BLASTN	967	1e-71	85
1757	16	701123056H1	SOYMON037	g1724103	BLASTN	968	1e-71	85
1758	16	700983479H1	SOYMON009	g726031	BLASTN	653	1e-70	85
1759	16	701063733H1	SOYMON034	g1655577	BLASTN	850	1e-70	85
1760	16	700738366H1	SOYMON012	g726031	BLASTN	946	1e-70	85
1761	16	700866319H1	SOYMON016	g1655575	BLASTN	947	1e-70	85
1762	16	700789013H2	SOYMON011	g1655577	BLASTN	948	1e-70	85
1763	16	700896080H1	SOYMON027	g609224	BLASTN	949	1e-70	87
1764	16	LIB3039-021-Q1-E1-F12	LIB3039	g16508	BLASTN	950	1e-70	83
1765	16	700898284H1	SOYMON027	g429105	BLASTN	950	1e-70	86
1766	16	700901743H1	SOYMON027	g609224	BLASTN	950	1e-70	86
1767	16	700831048H1	SOYMON019	g429105	BLASTN	951	1e-70	85
1768	16	701038107H1	SOYMON029	g726031	BLASTN	952	1e-70	87
1769	16	700559703H1	SOYMON001	g726031	BLASTN	954	1e-70	88
1770	16	700848817H1	SOYMON021	g609224	BLASTN	954	1e-70	84
1771	16	700896032H1	SOYMON027	g609224	BLASTN	956	1e-70	87
1772	16	700944764H1	SOYMON024	g16508	BLASTN	567	1e-69	85
1773	16	701011015H1	SOYMON019	g1724103	BLASTN	647	1e-69	81
1774	16	701125662H1	SOYMON037	g609224	BLASTN	751	1e-69	87
1775	16	701046895H1	SOYMON032	g16508	BLASTN	795	1e-69	83
1776	16	701012632H1	SOYMON019	g1655577	BLASTN	800	1e-69	85
1777	16	701005244H1	SOYMON019	g1724103	BLASTN	804	1e-69	82
1778	16	LIB3050-023-Q1-K1-H10	LIB3050	g609224	BLASTN	899	1e-69	83
1779	16	700892558H1	SOYMON024	g1655575	BLASTN	936	1e-69	85
1780	16	700988779H1	SOYMON011	g16508	BLASTN	937	1e-69	82
1781	16	701203923H2	SOYMON035	g429105	BLASTN	938	1e-69	86
1782	16	701010231H2	SOYMON019	g1724103	BLASTN	938	1e-69	83
1783	16	701041790H1	SOYMON029	g450548	BLASTN	939	1e-69	84
1784	16	700967887H1	SOYMON033	g1724103	BLASTN	940	1e-69	87
1785	16	701123361H1	SOYMON037	g16508	BLASTN	941	1e-69	84
1786	16	701123154H1	SOYMON037	g1724103	BLASTN	942	1e-69	86
1787	16	701045767H1	SOYMON032	g726031	BLASTN	942	1e-69	85
1788	16	700983288H1	SOYMON009	g609224	BLASTN	945	1e-69	83
1789	16	700653053H1	SOYMON003	g609224	BLASTN	945	1e-69	86
1790	16	701044226H1	SOYMON032	g1655577	BLASTN	486	1e-68	83
1791	16	700969927H1	SOYMON005	g497899	BLASTN	750	1e-68	85
1792	16	700547956H1	SOYMON001	g609224	BLASTN	779	1e-68	87
1793	16	LIB3049-048-Q1-E1-E4	LIB3049	g609224	BLASTN	827	1e-68	83



1794	16	701140780H1	SOYMON038	g1655577	BLASTN	922	1e-68	85
1795	16	700849166H1	SOYMON021	g1724103	BLASTN	923	1e-68	84
1796	16	700891945H1	SOYMON024	g609556	BLASTN	925	1e-68	87
1797	16	700658051H1	SOYMON004	g429105	BLASTN	925	1e-68	83
1798	16	700942415H1	SOYMON024	g1655575	BLASTN	928	1e-68	84
1799	16	701041890H1	SOYMON029	g1724103	BLASTN	930	1e-68	82
1800	16	LIB3028-006-Q1-B1-H12	LIB3028	g16508	BLASTN	931	1e-68	81
1801	16	700967039H1	SOYMON029	g429105	BLASTN	931	1e-68	85
1802	16	700836178H1	SOYMON019	g1724103	BLASTN	933	1e-68	85
1803	16	700897558H1	SOYMON027	g16508	BLASTN	737	1e-67	85
1804	16	LIB3050-004-Q1-E1-A2	LIB3050	g609224	BLASTN	796	1e-67	80
1805	16	700730236H1	SOYMON009	g726031	BLASTN	816	1e-67	84
1806	16	700833936H1	SOYMON019	g1724103	BLASTN	915	1e-67	84
1807	16	700897552H1	SOYMON027	g1655577	BLASTN	916	1e-67	84
1808	16	700945440H1	SOYMON024	g609556	BLASTN	917	1e-67	84
1809	16	700961368H1	SOYMON022	g169664	BLASTN	918	1e-67	88
1810	16	700789702H1	SOYMON011	g609224	BLASTN	921	1e-67	86
1811	16	700786541H1	SOYMON011	g429105	BLASTN	513	1e-66	84
1812	16	700987282H1	SOYMON009	g16508	BLASTN	523	1e-66	87
1813	16	700661002H1	SOYMON005	g2305013	BLASTN	836	1e-66	81
1814	16	701148312H1	SOYMON031	g862999	BLASTN	899	1e-66	84
1815	16	700738822H1	SOYMON012	g1655577	BLASTN	899	1e-66	85
1816	16	700940996H1	SOYMON024	g609556	BLASTN	901	1e-66	86
1817	16	700749195H1	SOYMON013	g609556	BLASTN	903	1e-66	81
1818	16	700893941H1	SOYMON024	g429105	BLASTN	903	1e-66	88
1819	16	700892888H1	SOYMON024	g609556	BLASTN	906	1e-66	86
1820	16	700901481H1	SOYMON027	g169664	BLASTN	638	1e-65	86
1821	16	700945269H1	SOYMON024	g169664	BLASTN	688	1e-65	86
1822	16	700746876H1	SOYMON013	g609556	BLASTN	740	1e-65	85
1823	16	700755043H1	SOYMON014	g609556	BLASTN	760	1e-65	87
1824	16	701097166H1	SOYMON028	g1655577	BLASTN	781	1e-65	82
1825	16	701129484H1	SOYMON037	g16508	BLASTN	898	1e-65	82
1826	16	700651014H1	SOYMON003	g167961	BLASTN	464	1e-64	81
1827	16	701134363H1	SOYMON038	g726031	BLASTN	687	1e-64	84
1828	16	701124677H1	SOYMON037	g726031	BLASTN	876	1e-64	85
1829	16	701000359H1	SOYMON018	g1724103	BLASTN	877	1e-64	87
1830	16	701139375H1	SOYMON038	g1724103	BLASTN	883	1e-64	84
1831	16	700832784H1	SOYMON019	g609224	BLASTN	883	1e-64	89
1832	16	700980227H1	SOYMON009	g1724103	BLASTN	884	1e-64	84
1833	16	700943177H1	SOYMON024	g1655577	BLASTN	885	1e-64	85
1834	16	700844446H1	SOYMON021	g2315139	BLASTN	355	1e-63	84
1835	16	700836453H1	SOYMON020	g862999	BLASTN	494	1e-63	85
1836	16	700983012H1	SOYMON009	g16508	BLASTN	756	1e-63	83
1837	16	700795805H1	SOYMON017	g1655577	BLASTN	833	1e-63	85
1838	16	70121366						

1848	16	LIB3027-010-Q1-B1-E12	LIB3027	g609224	BLASTN	854	1e-62	82
1849	16	701129389H1	SOYMON037	g16508	BLASTN	854	1e-62	83
1850	16	701045542H1	SOYMON032	g429105	BLASTN	855	1e-62	86
1851	16	700969901H1	SOYMON005	g726031	BLASTN	856	1e-62	84
1852	16	700984664H1	SOYMON009	g1724103	BLASTN	856	1e-62	81
1853	16	700561352H1	SOYMON002	g609224	BLASTN	858	1e-62	85
1854	16	701138828H1	SOYMON038	g16508	BLASTN	858	1e-62	84
1855	16	700845962H1	SOYMON021	g1655577	BLASTN	861	1e-62	87
1856	16	700896147H1	SOYMON027	g16508	BLASTN	336	1e-61	82
1857	16	701137929H1	SOYMON038	g1655577	BLASTN	502	1e-61	84
1858	16	LIB3040-032-Q1-E1-B8	LIB3040	g16508	BLASTN	521	1e-61	82
1859	16	701009537H1	SOYMON019	g726031	BLASTN	545	1e-61	86
1860	16	LIB3049-031-Q1-E1-E4	LIB3049	g609224	BLASTN	677	1e-61	82
1861	16	LIB3049-031-Q1-E1-C7	LIB3049	g167961	BLASTN	696	1e-61	82
1862	16	700891843H1	SOYMON024	g1655577	BLASTN	737	1e-61	84
1863	16	700561231H1	SOYMON002	g16508	BLASTN	839	1e-61	82
1864	16	700548238H1	SOYMON002	g429105	BLASTN	843	1e-61	85
1865	16	700901018H1	SOYMON027	g2305013	BLASTN	845	1e-61	82
1866	16	701134413H1	SOYMON038	g2305013	BLASTN	848	1e-61	82
1867	16	700653524H1	SOYMON003	g609224	BLASTN	499	1e-60	85
1868	16	LIB3049-017-Q1-E1-G8	LIB3049	g16508	BLASTN	514	1e-60	82
1869	16	701121077H1	SOYMON037	g16508	BLASTN	671	1e-60	84
1870	16	LIB3056-013-Q1-N1-A9	LIB3056	g609224	BLASTN	784	1e-60	86
1871	16	700943524H1	SOYMON024	g16508	BLASTN	831	1e-60	85
1872	16	700952889H1	SOYMON022	g429103	BLASTN	835	1e-60	84
1873	16	700730632H1	SOYMON009	g1655577	BLASTN	431	1e-59	82
1874	16	700649136H1	SOYMON003	g609224	BLASTN	486	1e-59	84
1875	16	700895591H1	SOYMON027	g609556	BLASTN	492	1e-59	87
1876	16	701009829H1	SOYMON019	g429103	BLASTN	572	1e-59	80
1877	16	LIB3039-011-Q1-E1-C11	LIB3039	g16508	BLASTN	619	1e-59	83
1878	16	LIB3040-055-Q1-E1-C4	LIB3040	g16508	BLASTN	622	1e-59	82
1879	16	701123571H1	SOYMON037	g2305013	BLASTN	682	1e-59	81
1880	16	LIB3049-004-Q1-E1-E5	LIB3049	g167961	BLASTN	771	1e-59	83
1881	16	701002239H1	SOYMON018	g609224	BLASTN	782	1e-59	86
1882	16	700971088H1	SOYMON005	g1724103	BLASTN	793	1e-59	81
1883	16	700864624H1	SOYMON016	g167961	BLASTN	817	1e-59	83
1884	16	700863534H1	SOYMON027	g16508	BLASTN	818	1e-59	86
1885	16	700749489H1	SOYMON013	g16508	BLASTN	823	1e-59	86
1886	16	700730689H1	SOYMON009	g16508	BLASTN	825	1e-59	83
1887	16	LIB3049-050-Q1-E1-A11	LIB3049	g16508	BLASTN	831	1e-59	83
1888	16	700850803H1	SOYMON023	g1655577	BLASTN	511	1e-58	83
1889	16	700992317H1	SOYMON011	g1655577	BLASTN	520	1e-58	79
1890	16	701119129H1	SOYMON037	g16508	BLASTN	553	1e-58	83
1891	16	700657623H1	SOYMON004	g1724103	BLASTN	600	1e-58	83
1892	16</							

1902	16	700902333H1	SOYMON027	g609224	BLASTN	700	1e-57	82
1903	16	LIB3039-017-Q1-E1-D9	LIB3039	g16508	BLASTN	703	1e-57	81
1904	16	LIB3040-026-Q1-E1-A4	LIB3040	g16508	BLASTN	709	1e-57	83
1905	16	701130101H1	SOYMON037	g1724103	BLASTN	791	1e-57	88
1906	16	700902482H1	SOYMON027	g169664	BLASTN	797	1e-57	81
1907	16	700730789H1	SOYMON009	g16508	BLASTN	798	1e-57	82
1908	16	700868670H1	SOYMON016	g16508	BLASTN	800	1e-57	83
1909	16	LIB3029-011-Q1-B1-D1	LIB3029	g609224	BLASTN	800	1e-57	82
1910	16	701119278H1	SOYMON037	g609224	BLASTN	801	1e-57	83
1911	16	700747731H1	SOYMON013	g16508	BLASTN	801	1e-57	86
1912	16	LIB3049-017-Q1-E1-A11	LIB3049	g16508	BLASTN	811	1e-57	80
1913	16	700890428H1	SOYMON024	g609224	BLASTN	482	1e-56	83
1914	16	700562326H1	SOYMON002	g609224	BLASTN	778	1e-56	82
1915	16	700567740H1	SOYMON002	g609224	BLASTN	781	1e-56	85
1916	16	701061514H1	SOYMON033	g1724103	BLASTN	784	1e-56	86
1917	16	701135670H1	SOYMON038	g429105	BLASTN	785	1e-56	81
1918	16	701139657H1	SOYMON038	g609224	BLASTN	786	1e-56	85
1919	16	701123371H1	SOYMON037	g16508	BLASTN	788	1e-56	86
1920	16	700838744H1	SOYMON020	g16508	BLASTN	789	1e-56	82
1921	16	701070458H1	SOYMON034	g1655577	BLASTN	457	1e-55	78
1922	16	701003437H1	SOYMON019	g429105	BLASTN	474	1e-55	83
1923	16	700835976H1	SOYMON019	g16508	BLASTN	562	1e-55	84
1924	16	700894535H1	SOYMON024	g16508	BLASTN	570	1e-55	86
1925	16	700752755H1	SOYMON014	g1655577	BLASTN	690	1e-55	83
1926	16	LIB3049-032-Q1-E1-C9	LIB3049	g16508	BLASTN	700	1e-55	83
1927	16	LIB3040-030-Q1-E1-B5	LIB3040	g16508	BLASTN	709	1e-55	84
1928	16	701136935H1	SOYMON038	g609224	BLASTN	766	1e-55	85
1929	16	700682088H1	SOYMON008	g169664	BLASTN	767	1e-55	90
1930	16	700682188H1	SOYMON008	g169664	BLASTN	767	1e-55	90
1931	16	701068649H1	SOYMON034	g16508	BLASTN	770	1e-55	83
1932	16	700726623H1	SOYMON009	g16508	BLASTN	771	1e-55	85
1933	16	700751129H1	SOYMON014	g16508	BLASTN	772	1e-55	86
1934	16	700945234H1	SOYMON024	g16508	BLASTN	773	1e-55	83
1935	16	701133507H2	SOYMON038	g609224	BLASTN	776	1e-55	85
1936	16	700902459H1	SOYMON027	g726031	BLASTN	777	1e-55	84
1937	16	700986624H1	SOYMON009	g16508	BLASTN	612	1e-54	83
1938	16	701097020H1	SOYMON028	g609224	BLASTN	615	1e-54	84
1939	16	701131409H1	SOYMON038	g16508	BLASTN	755	1e-54	91
1940	16	700750123H1	SOYMON013	g726031	BLASTN	756	1e-54	86
1941	16	701040251H1	SOYMON029	g16508	BLASTN	756	1e-54	85
1942	16	700732290H1	SOYMON010	g169664	BLASTN	758	1e-54	90
1943	16	701117458H1	SOYMON037	g609224	BLASTN	759	1e-54	82
1944	16	701118709H1	SOYMON037	g16508	BLASTN	760	1e-54	91
1945	16	701012834H1	SOYMON019	g16508	BLASTN	760	1e-54	91
1946	16	701133316H1	SOYMON038	g16508	BLASTN	760	1e-54	91
1947	16	701129646H1	SOYMON037	g16508	BLASTN	760	1e-54	91
1948	16	700732265H1	SOYMON010	g169664	BLASTN	760	1e-54	90
1949	16	701040796H1	SOYMON029	g1724103	BLASTN	760	1e-54	89
1950	16	700846350H1	SOYMON021	g16508	BLASTN	761	1e-54	86
1951	16	701137005H1	SOYMON038	g16508	BLASTN	761	1e-54	86
1952	16	701046506H1	SOYMON032	g16508	BLASTN	761	1e-54	91
1953	16	700894462H1	SOYMON024	g16508	BLASTN	761	1e-54	86
1954	16	700973847H1	SOYMON005	g16508	BLASTN	766	1e-54	87
1955	16	701128215H1	SOYMON037	g609556	BLASTN	466	1e-53	82

1956	16	700842468H1	SOYMON020	g429105	BLASTN	548	1e-53	86
1957	16	701051552H1	SOYMON032	g609224	BLASTN	744	1e-53	85
1958	16	700944049H1	SOYMON024	g609224	BLASTN	744	1e-53	85
1959	16	701120261H1	SOYMON037	g609224	BLASTN	746	1e-53	84
1960	16	700897524H1	SOYMON027	g609224	BLASTN	751	1e-53	84
1961	16	LIB3049-042-Q1-E1-F7	LIB3049	g450548	BLASTN	761	1e-53	75
1962	16	700896909H1	SOYMON027	g429105	BLASTN	536	1e-52	82
1963	16	700974820H1	SOYMON005	g1724103	BLASTN	582	1e-52	86
1964	16	701143224H1	SOYMON038	g609224	BLASTN	731	1e-52	84
1965	16	701010322H1	SOYMON019	g609224	BLASTN	731	1e-52	85
1966	16	701130510H1	SOYMON038	g609224	BLASTN	732	1e-52	83
1967	16	700724904H1	SOYMON009	g609224	BLASTN	733	1e-52	85
1968	16	701119845H1	SOYMON037	g609224	BLASTN	734	1e-52	85
1969	16	701107871H1	SOYMON036	g16508	BLASTN	737	1e-52	86
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1971	16	700896469H1	SOYMON027	g16508	BLASTN	741	1e-52	86
1972	16	700792178H1	SOYMON011	g16508	BLASTN	742	1e-52	85
1973	16	701099906H1	SOYMON028	g16508	BLASTN	431	1e-51	89
1974	16	701134724H2	SOYMON038	g16508	BLASTN	553	1e-51	85
1975	16	700749712H1	SOYMON013	g16508	BLASTN	689	1e-51	87
1976	16	700982567H1	SOYMON009	g609224	BLASTN	723	1e-51	85
1977	16	701013758H1	SOYMON019	g609224	BLASTN	723	1e-51	85
1978	16	700868508H1	SOYMON016	g609224	BLASTN	723	1e-51	85
1979	16	700749316H1	SOYMON013	g609224	BLASTN	723	1e-51	85
1980	16	701045367H1	SOYMON032	g16508	BLASTN	724	1e-51	91
1981	16	701205494H1	SOYMON035	g609224	BLASTN	724	1e-51	84
1982	16	700556784H1	SOYMON001	g16508	BLASTN	724	1e-51	91
1983	16	701099879H1	SOYMON028	g16508	BLASTN	726	1e-51	80
1984	16	701131671H1	SOYMON038	g609224	BLASTN	728	1e-51	85
1985	16	701011505H1	SOYMON019	g609224	BLASTN	728	1e-51	85
1986	16	701009973H2	SOYMON019	g609224	BLASTN	728	1e-51	85
1987	16	700793520H1	SOYMON017	g609224	BLASTN	728	1e-51	85
1988	16	700753622H1	SOYMON014	g609224	BLASTN	728	1e-51	85
1989	16	700984052H1	SOYMON009	g609224	BLASTN	728	1e-51	83
1990	16	701108521H1	SOYMON036	g609224	BLASTN	728	1e-51	85
1991	16	700957203H1	SOYMON022	g1724103	BLASTN	464	1e-50	86
1992	16	700554120H1	SOYMON001	g609224	BLASTN	528	1e-50	84
1993	16	700555954H1	SOYMON001	g167961	BLASTN	528	1e-50	86
1994	16	701124141H1	SOYMON037	g609224	BLASTN	537	1e-50	85
1995	16	701003232H1	SOYMON019	g16508	BLASTN	544	1e-50	88
1996	16	700653112H1	SOYMON003	g16508	BLASTN	559	1e-50	91
1997	16	701103353H1	SOYMON028	g1655577	BLASTN	581	1e-50	84
1998	16	700562790H1	SOYMON002	g497899	BLASTN	708	1e-50	86
1999	16	701097303H1	SOYMON028	g609224	BLASTN	711	1e-50	84
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2010	16	700745426H1	SOYMON013	g16508	BLASTN	697	1e-49	84
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2012	16	701003787H1	SOYMON019	g609224	BLASTN	697	1e-49	85
2013	16	700840830H1	SOYMON020	g1724103	BLASTN	699	1e-49	79
2014	16	700901588H1	SOYMON027	g609224	BLASTN	702	1e-49	85
2015	16	701037824H1	SOYMON029	g497899	BLASTN	702	1e-49	87
2016	16	701131888H1	SOYMON038	g609224	BLASTN	702	1e-49	85
2017	16	701009947H2	SOYMON019	g609224	BLASTN	702	1e-49	85
2018	16	700729216H1	SOYMON009	g497899	BLASTN	702	1e-49	87
2019	16	700831705H1	SOYMON019	g609224	BLASTN	702	1e-49	85
2020	16	700900329H1	SOYMON027	g429105	BLASTN	705	1e-49	88
2021	16	700563946H1	SOYMON002	g16508	BLASTN	705	1e-49	83
2022	16	700808370H1	SOYMON024	g609556	BLASTN	459	1e-48	83
2023	16	LIB3040-001-Q1-E1-H4	LIB3040	g1724103	BLASTN	475	1e-48	76
2024	16	700989482H1	SOYMON011	g16508	BLASTN	498	1e-48	86
2025	16	701001311H1	SOYMON018	g16508	BLASTN	655	1e-48	85
2026	16	700734733H1	SOYMON010	g497899	BLASTN	682	1e-48	87
2027	16	701013070H1	SOYMON019	g497899	BLASTN	682	1e-48	87
2028	16	701120989H1	SOYMON037	g16508	BLASTN	683	1e-48	91
2029	16	LIB3049-025-Q1-E1-B4	LIB3049	g167961	BLASTN	687	1e-48	82
2030	16	700808455H1	SOYMON024	g16508	BLASTN	688	1e-48	87
2031	16	701101319H1	SOYMON028	g16508	BLASTN	688	1e-48	91
2032	16	701037087H1	SOYMON029	g609224	BLASTN	689	1e-48	85
2033	16	701205225H1	SOYMON035	g16508	BLASTN	689	1e-48	84
2034	16	701136807H1	SOYMON038	g609224	BLASTN	690	1e-48	85
2035	16	701118459H1	SOYMON037	g609224	BLASTN	690	1e-48	85
2036	16	700942825H1	SOYMON024	g16508	BLASTN	691	1e-48	85
2037	16	701139796H1	SOYMON038	g497899	BLASTN	692	1e-48	87
2038	16	700557040H1	SOYMON001	g609224	BLASTN	692	1e-48	85
2039	16	701015715H1	SOYMON038	g497899	BLASTN	692	1e-48	87
2040	16	700726424H1	SOYMON009	g497899	BLASTN	693	1e-48	87
2041	16	700790811H1	SOYMON011	g497899	BLASTN	693	1e-48	87
2042	16	LIB3040-038-Q1-E1-E5	LIB3040	g16508	BLASTN	709	1e-48	85
2043	16	700896626H1	SOYMON027	g429107	BLASTN	430	1e-47	84
2044	16	700562158H1	SOYMON002	g609224	BLASTN	518	1e-47	84
2045	16	700747095H1	SOYMON013	g16508	BLASTN	538	1e-47	87
2046	16	700726532H1	SOYMON009	g609224	BLASTN	622	1e-47	85
2047	16	701137686H1	SOYMON038	g497899	BLASTN	671	1e-47	85
2048	16	701009148H1	SOYMON019	g16508	BLASTN	673	1e-47	91
2049	16	701048279H1	SOYMON032	g16508	BLASTN	674	1e-47	86
2050	16	701120185H1	SOYMON037	g16508	BLASTN	674	1e-47	91
2051	16	701040355H1	SOYMON029	g497899	BLASTN	675	1e-47	86
2052	16	701130203H1	SOYMON037	g609224	BLASTN	678	1e-47	83
2053	16	701015794H1	SOYMON038	g16508	BLASTN	681	1e-47	91
2054	16	700732181H1	SOYMON010	g16508	BLASTN	681	1e-47	86
2055	16	LIB3050-018-Q1-E1-D10	LIB3050	g2305013	BLASTN	696	1e-47	81
2056	16	700899157H1	SOYMON027	g609224	BLASTN	486	1e-46	84
2057	16	701119905H1	SOYMON037	g497899	BLASTN	507	1e-46	86
2058	16	701062873H1	SOYMON033	g497899	BLASTN	509	1e-46	88
2059	16	701210886H1	SOYMON035	g16508	BLASTN	540	1e-46	84
2060	16	700893278H1	SOYMON024	g16508	BLASTN	542	1e-46	77
2061	16	701036970H1	SOYMON029	g16508	BLASTN	579	1e-46	88
2062	16	700901932H1	SOYMON027	g609224	BLASTN	640	1e-46	85
2063	16	701044214H1	SOYMON032	g429105	BLASTN	658	1e-46	85

2064	16	701056917H1	SOYMON033	g16508	BLASTN	661	1e-46	85
2065	16	700981560H1	SOYMON009	g497899	BLASTN	661	1e-46	87
2066	16	701213107H1	SOYMON035	g609224	BLASTN	661	1e-46	83
2067	16	700834235H1	SOYMON019	g497899	BLASTN	661	1e-46	87
2068	16	700749413H1	SOYMON013	g16508	BLASTN	662	1e-46	85
2069	16	700889148H1	SOYMON024	g16508	BLASTN	662	1e-46	85
2070	16	700745009H1	SOYMON013	g609224	BLASTN	662	1e-46	85
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2072	16	701131927H1	SOYMON038	g497899	BLASTN	666	1e-46	87
2073	16	701119069H1	SOYMON037	g497899	BLASTN	666	1e-46	87
2074	16	701106908H1	SOYMON036	g497899	BLASTN	666	1e-46	87
2075	16	701103063H1	SOYMON028	g497899	BLASTN	666	1e-46	87
2076	16	700686659H1	SOYMON008	g166873	BLASTN	667	1e-46	86
2077	16	701040620H1	SOYMON029	g16508	BLASTN	669	1e-46	86
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2079	16	700957508H1	SOYMON022	g1724103	BLASTN	669	1e-46	82
2080	16	701008548H1	SOYMON019	g497899	BLASTN	500	1e-45	87
2081	16	700978303H1	SOYMON009	g497899	BLASTN	506	1e-45	87
2082	16	701119990H1	SOYMON037	g497899	BLASTN	512	1e-45	87
2083	16	700646222H1	SOYMON012	g16508	BLASTN	526	1e-45	85
2084	16	701040631H1	SOYMON029	g609556	BLASTN	542	1e-45	87
2085	16	700894009H1	SOYMON024	g497899	BLASTN	646	1e-45	87
2086	16	700908702H1	SOYMON022	g609224	BLASTN	646	1e-45	85
2087	16	700838642H1	SOYMON020	g609224	BLASTN	646	1e-45	85
2088	16	700832089H1	SOYMON019	g609224	BLASTN	646	1e-45	85
2089	16	700978279H1	SOYMON009	g609224	BLASTN	647	1e-45	80
2090	16	700555058H1	SOYMON001	g16508	BLASTN	649	1e-45	84
2091	16	700906478H1	SOYMON022	g497899	BLASTN	651	1e-45	87
2092	16	700897237H1	SOYMON027	g16508	BLASTN	653	1e-45	86
2093	16	701125786H1	SOYMON037	g16508	BLASTN	654	1e-45	84
2094	16	700562836H1	SOYMON002	g16508	BLASTN	655	1e-45	84
2095	16	700665969H1	SOYMON005	g497899	BLASTN	656	1e-45	87
2096	16	701009838H1	SOYMON019	g497899	BLASTN	656	1e-45	87
2097	16	701211770H1	SOYMON035	g16508	BLASTN	657	1e-45	84
2098	16	700654550H1	SOYMON004	g16508	BLASTN	657	1e-45	83
2099	16	700567949H1	SOYMON002	g16508	BLASTN	657	1e-45	84
2100	16	701048175H1	SOYMON032	g16508	BLASTN	658	1e-45	91
2101	16	700901037H1	SOYMON027	g16508	BLASTN	658	1e-45	86
2102	16	700656917H1	SOYMON004	g1724103	BLASTN	407	1e-44	81
2103	16	700565684H1	SOYMON002	g16508	BLASTN	463	1e-44	86
2104	16	701002808H1	SOYMON019	g497899	BLASTN	503	1e-44	87
2105	16	701107115H1	SOYMON036	g497899	BLASTN	507	1e-44	87
2106	16	701122669H1	SOYMON037	g16508	BLASTN	636	1e-44	82
2107	16	700894409H1	SOYMON024	g497899	BLASTN	636	1e-44	87
2108	16	700848374H1	SOYMON021	g497899	BLASTN	636	1e-44	87
2109	16	700902420H1	SOYMON027	g497899	BLASTN	636	1e-44	87
2110	16	700747450H1	SOYMON013	g16508	BLASTN	638	1e-44	91
2111	16	701125869H1	SOYMON037	g16508	BLASTN	638	1e-44	91
2112	16	700731302H1	SOYMON010	g16508	BLASTN	639	1e-44	85
2113	16	701099068H1	SOYMON028	g2305013	BLASTN	640	1e-44	81
2114	16	700975556H1	SOYMON009	g450548	BLASTN	641	1e-44	84
2115	16	700889660H1	SOYMON024	g16508	BLASTN	642	1e-44	86
2116	16	700951744H1	SOYMON022	g16508	BLASTN	642	1e-44	86
2117	16	700750251H1	SOYMON013	g609224	BLASTN	643	1e-44	86

2118	16	700834611H1	SOYMON019	g16508	BLASTN	643	1e-44	91
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2120	16	700673803H1	SOYMON007	g16508	BLASTN	644	1e-44	84
2121	16	700958721H1	SOYMON022	g16508	BLASTN	645	1e-44	86
2122	16	700985717H1	SOYMON009	g16508	BLASTN	646	1e-44	82
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2125	16	700987656H1	SOYMON009	g497899	BLASTN	476	1e-43	87
2126	16	700755957H1	SOYMON014	g497899	BLASTN	483	1e-43	86
2127	16	700749331H1	SOYMON013	g2305013	BLASTN	521	1e-43	84
2128	16	700830984H1	SOYMON019	g497899	BLASTN	622	1e-43	86
2129	16	700906176H1	SOYMON022	g16508	BLASTN	623	1e-43	86
2130	16	701100070H2	SOYMON028	g16508	BLASTN	623	1e-43	91
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2133	16	700976710H1	SOYMON009	g2305013	BLASTN	625	1e-43	79
2134	16	701117925H2	SOYMON037	g16508	BLASTN	625	1e-43	84
2135	16	700748825H1	SOYMON013	g16508	BLASTN	626	1e-43	84
2136	16	700899651H1	SOYMON027	g16508	BLASTN	626	1e-43	84
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2139	16	700754423H1	SOYMON014	g16508	BLASTN	628	1e-43	91
2140	16	700760701H1	SOYMON015	g16508	BLASTN	628	1e-43	83
2141	16	701098124H1	SOYMON028	g2305013	BLASTN	628	1e-43	78
2142	16	700984979H1	SOYMON009	g16508	BLASTN	628	1e-43	91
2143	16	700565262H1	SOYMON002	g497899	BLASTN	629	1e-43	86
2144	16	700954979H1	SOYMON022	g497899	BLASTN	630	1e-43	87
2145	16	700834601H1	SOYMON019	g497899	BLASTN	630	1e-43	87
2146	16	700953156H1	SOYMON022	g609224	BLASTN	630	1e-43	85
2147	16	700865217H1	SOYMON016	g16508	BLASTN	631	1e-43	77
2148	16	700852947H1	SOYMON023	g16508	BLASTN	631	1e-43	84
2149	16	700943547H1	SOYMON024	g497899	BLASTN	631	1e-43	86
2150	16	701139277H1	SOYMON038	g609224	BLASTN	632	1e-43	82
2151	16	700900155H1	SOYMON027	g429105	BLASTN	264	1e-42	81
2152	16	700970581H1	SOYMON005	g497899	BLASTN	296	1e-42	86
2153	16	700654232H1	SOYMON003	g497899	BLASTN	348	1e-42	88
2154	16	700743608H1	SOYMON012	g497899	BLASTN	354	1e-42	86
2155	16	700986620H1	SOYMON009	g609224	BLASTN	360	1e-42	84
2156	16	700562590H1	SOYMON002	g16508	BLASTN	476	1e-42	83
2157	16	701119690H1	SOYMON037	g167961	BLASTN	506	1e-42	84
2158	16	700951730H1	SOYMON022	g497899	BLASTN	507	1e-42	87
2159	16	701004525H1	SOYMON019	g16508	BLASTN	516	1e-42	91
2160	16	700892637H1	SOYMON024	g16508	BLASTN	523	1e-42	81
2161	16	700560679H1	SOYMON001	g16508	BLASTN	526	1e-42	83
2162	16	700897888H1	SOYMON027	g16508	BLASTN			

2172	16	701015704H1	SOYMON038	g16508	BLASTN	620	1e-42	85
2173	16	700966710H1	SOYMON028	g16508	BLASTN	620	1e-42	83
2174	16	701004268H1	SOYMON019	g16508	BLASTN	620	1e-42	86
2175	16	701138901H1	SOYMON038	g497899	BLASTN	285	1e-41	85
2176	16	700648891H1	SOYMON003	g16508	BLASTN	481	1e-41	84
2177	16	700741421H1	SOYMON012	g609224	BLASTN	511	1e-41	82
2178	16	700555653H1	SOYMON001	g166873	BLASTN	512	1e-41	85
2179	16	700958314H1	SOYMON022	g497899	BLASTN	599	1e-41	87
2180	16	700960055H1	SOYMON022	g497899	BLASTN	599	1e-41	87
2181	16	700901925H1	SOYMON027	g16508	BLASTN	599	1e-41	84
2182	16	700741987H1	SOYMON012	g497899	BLASTN	599	1e-41	87
2183	16	700662837H1	SOYMON005	g497899	BLASTN	599	1e-41	87
2184	16	701060927H1	SOYMON033	g497899	BLASTN	599	1e-41	87
2185	16	700562973H1	SOYMON002	g16508	BLASTN	601	1e-41	87
2186	16	700754586H1	SOYMON014	g16508	BLASTN	603	1e-41	84
2187	16	701132674H1	SOYMON038	g16508	BLASTN	604	1e-41	85
2188	16	701010012H2	SOYMON019	g609224	BLASTN	604	1e-41	84
2189	16	701123643H1	SOYMON037	g16508	BLASTN	604	1e-41	85
2190	16	700894196H1	SOYMON024	g16508	BLASTN	604	1e-41	85
2191	16	700899248H1	SOYMON027	g16508	BLASTN	604	1e-41	85
2192	16	701207680H1	SOYMON035	g16508	BLASTN	605	1e-41	84
2193	16	700899210H1	SOYMON027	g16508	BLASTN	605	1e-41	84
2194	16	700898762H1	SOYMON027	g16508	BLASTN	605	1e-41	87
2195	16	700983636H1	SOYMON009	g497899	BLASTN	606	1e-41	87
2196	16	700845511H1	SOYMON021	g16508	BLASTN	606	1e-41	89
2197	16	700563416H1	SOYMON002	g16508	BLASTN	606	1e-41	83
2198	16	701004041H1	SOYMON019	g16508	BLASTN	607	1e-41	84
2199	16	701097184H1	SOYMON028	g16508	BLASTN	607	1e-41	87
2200	16	700750691H1	SOYMON014	g609224	BLASTN	608	1e-41	84
2201	16	701208091H1	SOYMON035	g16508	BLASTN	608	1e-41	84
2202	16	700973888H1	SOYMON005	g609224	BLASTN	609	1e-41	85
2203	16	700831972H1	SOYMON019	g16508	BLASTN	609	1e-41	85
2204	16	701010090H2	SOYMON019	g16508	BLASTN	610	1e-41	87
2205	16	700890067H1	SOYMON024	g16508	BLASTN	610	1e-41	87
2206	16	700833227H1	SOYMON019	g16508	BLASTN	610	1e-41	85
2207	16	700563171H1	SOYMON002	g16508	BLASTN	370	1e-40	90
2208	16	701121457H1	SOYMON037	g609224	BLASTN	372	1e-40	85
2209	16	700566851H1	SOYMON002	g16508	BLASTN	402	1e-40	82
2210	16	700753221H1	SOYMON014	g609224	BLASTN	422	1e-40	84
2211	16	700749039H1	SOYMON013	g16508	BLASTN	475	1e-40	84
2212	16	700558940H1	SOYMON001	g16508	BLASTN	493	1e-40	80
2213	16	700757695H1	SOYMON015	g16508	BLASTN	526	1e-40	83
2214	16	701102262H1	SOYMON028	g2305013	BLASTN	587	1e-40	81
2215	16	700747484H1	SOYMON013	g16508	BLASTN	587	1e-40	85
2216	16	700891875H1	SOYMON024	g16508	BLASTN	587	1e-40	85
2217	16	700894439H1	SOYMON024	g497899	BLASTN	588	1e-40	88
2218	16	701062577H1	SOYMON033	g16508	BLASTN	588	1e-40	87
2219	16	700964366H1	SOYMON022	g16508	BLASTN	589	1e-40	85
2220	16	701210016H1	SOYMON035	g16508	BLASTN	589	1e-40	83
2221	16	700901573H1	SOYMON027	g16508	BLASTN	589	1e-40	85
2222	16	700750533H1	SOYMON014	g16508	BLASTN	589	1e-40	85
2223	16	700888820H1	SOYMON024	g497899	BLASTN	589	1e-40	87
2224	16	700891965H1	SOYMON024	g497899	BLASTN	589	1e-40	87
2225	16	700865001H1	SOYMON016	g16508	BLASTN	589	1e-40	85



2226	16	700654923H1	SOYMON004	g16508	BLASTN	590	1e-40	84
2227	16	700746169H1	SOYMON013	g16508	BLASTN	590	1e-40	84
2228	16	700745792H1	SOYMON013	g16508	BLASTN	591	1e-40	85
2229	16	700966953H1	SOYMON029	g16508	BLASTN	591	1e-40	85
2230	16	701141657H1	SOYMON038	g16508	BLASTN	592	1e-40	86
2231	16	700943078H1	SOYMON024	g16508	BLASTN	598	1e-40	85
2232	16	701137073H1	SOYMON038	g16508	BLASTN	598	1e-40	90
2233	16	700966730H1	SOYMON028	g16508	BLASTN	598	1e-40	84
2234	16	701137731H1	SOYMON038	g16508	BLASTN	371	1e-39	84
2235	16	701044050H1	SOYMON032	g497899	BLASTN	428	1e-39	89
2236	16	700993309H1	SOYMON011	g497899	BLASTN	434	1e-39	88
2237	16	700874483H1	SOYMON018	g497899	BLASTN	434	1e-39	87
2238	16	701014866H1	SOYMON019	g16508	BLASTN	454	1e-39	89
2239	16	700894603H1	SOYMON024	g16508	BLASTN	454	1e-39	85
2240	16	700958677H1	SOYMON022	g497899	BLASTN	455	1e-39	87
2241	16	700754001H1	SOYMON014	g609224	BLASTN	518	1e-39	85
2242	16	700946430H1	SOYMON024	g497899	BLASTN	578	1e-39	87
2243	16	700729892H1	SOYMON009	g497899	BLASTN	578	1e-39	87
2244	16	700753230H1	SOYMON014	g497899	BLASTN	578	1e-39	87
2245	16	700562584H1	SOYMON002	g16508	BLASTN	578	1e-39	82
2246	16	700842880H1	SOYMON020	g16508	BLASTN	580	1e-39	82
2247	16	700852561H1	SOYMON023	g16508	BLASTN	582	1e-39	85
2248	16	700941290H1	SOYMON024	g497899	BLASTN	583	1e-39	87
2249	16	700750361H1	SOYMON013	g497899	BLASTN	584	1e-39	80
2250	16	700841915H1	SOYMON020	g16508	BLASTN	585	1e-39	85
2251	16	700944462H1	SOYMON024	g16508	BLASTN	585	1e-39	85
2252	16	700548174H1	SOYMON002	g16508	BLASTN	342	1e-38	82
2253	16	700942324H1	SOYMON024	g497899	BLASTN	403	1e-38	87
2254	16	700788450H1	SOYMON011	g497899	BLASTN	416	1e-38	86
2255	16	701127954H1	SOYMON037	g16508	BLASTN	449	1e-38	76
2256	16	700742223H1	SOYMON012	g1724103	BLASTN	451	1e-38	77
2257	16	701102722H1	SOYMON028	g16508	BLASTN	466	1e-38	92
2258	16	701046957H1	SOYMON032	g16508	BLASTN	517	1e-38	85
2259	16	700836169H1	SOYMON019	g497899	BLASTN	517	1e-38	87
2260	16	700697967H1	SOYMON015	g16508	BLASTN	521	1e-38	84
2261	16	700678867H1	SOYMON007	g609224	BLASTN	566	1e-38	82
2262	16	700838753H1	SOYMON020	g16508	BLASTN	567	1e-38	85
2263	16	700726468H1	SOYMON009	g16508	BLASTN	570	1e-38	85
2264	16	701014631H1	SOYMON019	g497899	BLASTN	571	1e-38	87
2265	16	700728719H1	SOYMON009	g497899	BLASTN	573	1e-38	87
2266	16	700962582H1	SOYMON022	g497899	BLASTN	573	1e-38	87
2267	16	701123183H1	SOYMON037	g16508	BLASTN	573	1e-38	84
2268	16	700752347H1	SOYMON014	g16508	BLASTN	574	1e-38	87
2269	16	701103315H1	SOYMON028	g16508	BLASTN	394	1e-37	86
2270	16	700750955H1	SOYMON014	g497899	BLASTN	414	1e-37	88
2271	16	700867493H1	SOYMON016	g609224	BLASTN	433	1e-37	85
2272	16	701054760H1	SOYMON032	g16508	BLASTN	475	1e-37	85
2273	16	700900903H1	SOYMON027	g16508	BLASTN	501	1e-37	84
2274	16	701102294H1	SOYMON028	g16508	BLASTN	502	1e-37	87
2275	16	701124034H1	SOYMON037	g16508	BLASTN	512	1e-37	82
2276	16	700981476H1	SOYMON009	g16508	BLASTN	526	1e-37	83
2277	16	700903712H1	SOYMON022	g609224	BLASTN	552	1e-37	85
2278	16	700648751H1	SOYMON003	g1655577	BLASTN	554	1e-37	86
2279	16	700829930H1	SOYMON019	g609224	BLASTN	556	1e-37	84

2280	16	700758078H1	SOYMON015	g16508	BLASTN	556	1e-37	84
2281	16	700754248H1	SOYMON014	g16508	BLASTN	557	1e-37	81
2282	16	700834650H1	SOYMON019	g609224	BLASTN	557	1e-37	85
2283	16	700746279H1	SOYMON013	g16508	BLASTN	558	1e-37	82
2284	16	700869124H1	SOYMON016	g16508	BLASTN	559	1e-37	83
2285	16	701100219H1	SOYMON028	g2305013	BLASTN	560	1e-37	80
2286	16	700992589H1	SOYMON011	g497899	BLASTN	560	1e-37	80
2287	16	700865642H1	SOYMON016	g16508	BLASTN	562	1e-37	84
2288	16	701068835H1	SOYMON034	g429105	BLASTN	321	1e-36	86
2289	16	700746908H1	SOYMON013	g16508	BLASTN	373	1e-36	81
2290	16	700954719H1	SOYMON022	g16508	BLASTN	475	1e-36	86
2291	16	701042790H1	SOYMON029	g497899	BLASTN	489	1e-36	88
2292	16	700835259H1	SOYMON019	g497899	BLASTN	507	1e-36	88
2293	16	700565816H1	SOYMON002	g16508	BLASTN	519	1e-36	82
2294	16	700895811H1	SOYMON027	g609224	BLASTN	540	1e-36	83
2295	16	700975709H1	SOYMON009	g609224	BLASTN	541	1e-36	84
2296	16	700751645H1	SOYMON014	g16508	BLASTN	542	1e-36	85
2297	16	700567087H1	SOYMON002	g16508	BLASTN	542	1e-36	85
2298	16	700893027H1	SOYMON024	g609224	BLASTN	542	1e-36	85
2299	16	700891185H1	SOYMON024	g609224	BLASTN	543	1e-36	84
2300	16	700851376H1	SOYMON023	g16508	BLASTN	543	1e-36	83
2301	16	700898322H1	SOYMON027	g609224	BLASTN	547	1e-36	85
2302	16	700762881H1	SOYMON015	g16508	BLASTN	323	1e-35	85
2303	16	700960023H1	SOYMON022	g16508	BLASTN	370	1e-35	90
2304	16	701136486H1	SOYMON038	g497899	BLASTN	378	1e-35	85
2305	16	700987688H1	SOYMON009	g497899	BLASTN	387	1e-35	83
2306	16	700836508H1	SOYMON020	g16508	BLASTN	431	1e-35	85
2307	16	700990779H1	SOYMON011	g167961	BLASTN	471	1e-35	86
2308	16	700753019H1	SOYMON014	g497899	BLASTN	476	1e-35	83
2309	16	701138490H1	SOYMON038	g16508	BLASTN	481	1e-35	83
2310	16	700751771H1	SOYMON014	g609224	BLASTN	528	1e-35	84
2311	16	701048324H1	SOYMON032	g609224	BLASTN	529	1e-35	84
2312	16	700835739H1	SOYMON019	g16508	BLASTN	532	1e-35	85
2313	16	700986295H1	SOYMON009	g497899	BLASTN	533	1e-35	86
2314	16	701205693H1	SOYMON035	g16508	BLASTN	535	1e-35	84
2315	16	700865674H1	SOYMON016	g609224	BLASTN	536	1e-35	85
2316	16	700943777H1	SOYMON024	g16508	BLASTN	536	1e-35	84
2317	16	700968207H1	SOYMON035	g609224	BLASTN	536	1e-35	85
2318	16	700898370H1	SOYMON027	g609224	BLASTN	536	1e-35	85
2319	16	700987890H1	SOYMON009	g609224	BLASTN	536	1e-35	85
2320	16	700896016H1	SOYMON027	g609224	BLASTN	536	1e-35	85
2321	16	700554440H1	SOYMON001	g16508	BLASTN	283	1e-34	82
2322	16	700789855H2	SOYMON011	g16508	BLASTN	293	1e-34	91
2323	16	701101393H1	SOYMON028	g16508	BLASTN	311	1e-34	84
2324	16	700946335H1	SOYMON024	g16508	BLASTN	323	1e-34	86
2325	16	701207420H1	SOYMON035	g169664	BLASTN	350	1e-34	88
2326	16	700733034H1	SOYMON010	g16508	BLASTN	384	1e-34	81
2327	16	700790653H2	SOYMON011	g497899	BLASTN	399	1e-34	81
2328	16	700901808H1	SOYMON027	g497899	BLASTN	430	1e-34	81
2329	16	700760954H1	SOYMON015	g609224	BLASTN	514	1e-34	84
2330	16	700896206H1	SOYMON027	g16508	BLASTN	516	1e-34	85
2331	16	701039157H1	SOYMON029	g609224	BLASTN	520	1e-34	76
2332	16	700991056H1	SOYMON011	g166873	BLASTN	520	1e-34	80
2333	16	701097173H1	SOYMON028	g609224	BLASTN	521	1e-34	85

2334	16	700900989H1	SOYMON027	g497899	BLASTN	521	1e-34	86
2335	16	700895584H1	SOYMON027	g609224	BLASTN	521	1e-34	85
2336	16	700726668H1	SOYMON009	g16508	BLASTN	521	1e-34	85
2337	16	701011191H1	SOYMON019	g16508	BLASTN	521	1e-34	85
2338	16	701100827H1	SOYMON028	g16508	BLASTN	522	1e-34	84
2339	16	701156732H1	SOYMON031	g16508	BLASTN	522	1e-34	84
2340	16	700889752H1	SOYMON024	g609224	BLASTN	523	1e-34	84
2341	16	701105873H1	SOYMON036	g16508	BLASTN	524	1e-34	87
2342	16	700958346H1	SOYMON022	g497899	BLASTN	525	1e-34	84
2343	16	700962972H1	SOYMON022	g609224	BLASTN	525	1e-34	84
2344	16	701055918H1	SOYMON032	g16508	BLASTN	526	1e-34	85
2345	16	701123061H1	SOYMON037	g16508	BLASTN	526	1e-34	85
2346	16	701109796H1	SOYMON036	g166873	BLASTN	335	1e-33	86
2347	16	701207350H1	SOYMON035	g497899	BLASTN	349	1e-33	89
2348	16	700835692H1	SOYMON019	g16508	BLASTN	377	1e-33	81
2349	16	700729083H1	SOYMON009	g16508	BLASTN	448	1e-33	85
2350	16	LIB3040-049-Q1-E1-E8	LIB3040	g16508	BLASTN	464	1e-33	80
2351	16	700896567H1	SOYMON027	g497899	BLASTN	502	1e-33	81
2352	16	700668032H1	SOYMON006	g609224	BLASTN	504	1e-33	85
2353	16	700895062H1	SOYMON024	g609224	BLASTN	505	1e-33	83
2354	16	700961178H1	SOYMON022	g16508	BLASTN	506	1e-33	84
2355	16	701202590H1	SOYMON035	g497899	BLASTN	508	1e-33	86
2356	16	700754960H1	SOYMON014	g16508	BLASTN	512	1e-33	83
2357	16	700748584H1	SOYMON013	g16508	BLASTN	512	1e-33	84
2358	16	700791669H1	SOYMON011	g609224	BLASTN	512	1e-33	84
2359	16	701210405H1	SOYMON035	g16508	BLASTN	526	1e-33	83
2360	16	LIB3040-054-Q1-E1-D8	LIB3040	g16508	BLASTN	531	1e-33	80
2361	16	700791176H1	SOYMON011	g497899	BLASTN	311	1e-32	87
2362	16	LIB3049-029-Q1-E1-C5	LIB3049	g167961	BLASTN	368	1e-32	73
2363	16	700893458H1	SOYMON024	g497899	BLASTN	380	1e-32	87
2364	16	700829728H1	SOYMON019	g497899	BLASTN	490	1e-32	88
2365	16	700833137H1	SOYMON019	g609224	BLASTN	490	1e-32	85
2366	16	701204592H2	SOYMON035	g609224	BLASTN	493	1e-32	86
2367	16	700834821H1	SOYMON019	g609224	BLASTN	494	1e-32	78
2368	16	700757167H1	SOYMON015	g16508	BLASTN	494	1e-32	85
2369	16	701203730H2	SOYMON035	g16508	BLASTN	496	1e-32	82
2370	16	701044359H1	SOYMON032	g609224	BLASTN	497	1e-32	84
2371	16	701010095H2	SOYMON019	g16508	BLASTN	498	1e-32	86
2372	16	701100059H2	SOYMON028	g16508	BLASTN	499	1e-32	80
2373	16	700844256H1	SOYMON021	g16508	BLASTN	500	1e-32	80
2374	16	700968296H1	SOYMON035	g497899	BLASTN	501	1e-32	85
2375	16	701110404H1	SOYMON036	g16508	BLASTN	333	1e-31	84
2376	16	700869025H1	SOYMON016	g497899	BLASTN	340	1e-31	86
2377	16	700556245H1	SOYMON001	g609224	BLASTN	355	1e-31	84
2378	16	700566166H1	SOYMON002	g726031	BLASTN	402	1e-31	77
2379	16	700941481H1	SOYMON024	g497899	BLASTN	423	1e-31	79
2380	16	700896940H1	SOYMON027	g169664	BLASTN	477	1e-31	85
2381	16	701145429H1	SOYMON031	g609224	BLASTN	478	1e-31	85
2382	16	700946496H1	SOYMON024	g609224	BLASTN	488	1e-31	86
2383	16	700902149H1	SOYMON027	g1655577	BLASTN	488	1e-31	87
2384	16	700653072H1	SOYMON003	g497899	BLASTN	489	1e-31	88
2385	16	701000261H1	SOYMON018	g16508	BLASTN	495	1e-31	78
2386	16	701039358H1	SOYMON029	g609224	BLASTN	320	1e-30	85
2387	16	701156951H1	SOYMON031	g609224	BLASTN	467	1e-30	86

2388	16	701040880H1	SOYMON029	g497899	BLASTN	477	1e-30	89
2389	16	700979632H2	SOYMON009	g609224	BLASTN	490	1e-30	83
2390	16	700983678H1	SOYMON009	g16508	BLASTN	192	1e-29	86
2391	16	700893644H1	SOYMON024	g609224	BLASTN	320	1e-29	85
2392	16	700962437H1	SOYMON022	g609224	BLASTN	364	1e-29	83
2393	16	701150545H1	SOYMON031	g16508	BLASTN	457	1e-29	86
2394	16	700555403H1	SOYMON001	g16508	BLASTN	458	1e-29	90
2395	16	700794307H1	SOYMON017	g16508	BLASTN	460	1e-29	87
2396	16	701137971H1	SOYMON038	g16508	BLASTN	460	1e-29	84
2397	16	700893774H1	SOYMON024	g609224	BLASTN	461	1e-29	86
2398	16	700565272H1	SOYMON002	g609224	BLASTN	464	1e-29	74
2399	16	700667161H1	SOYMON006	g16508	BLASTN	468	1e-29	75
2400	16	700752952H1	SOYMON014	g609224	BLASTN	279	1e-28	78
2401	16	700901534H1	SOYMON027	g2305013	BLASTN	288	1e-28	85
2402	16	701006617H1	SOYMON019	g497899	BLASTN	358	1e-28	79
2403	16	700753459H1	SOYMON014	g609224	BLASTN	446	1e-28	81
2404	16	701157089H1	SOYMON031	g609224	BLASTN	448	1e-28	84
2405	16	700831271H1	SOYMON019	g16508	BLASTN	453	1e-28	87
2406	16	701207325H1	SOYMON035	g167961	BLASTN	251	1e-27	81
2407	16	700566121H1	SOYMON002	g16508	BLASTN	259	1e-27	75
2408	16	701137878H1	SOYMON038	g609224	BLASTN	280	1e-27	85
2409	16	700763957H1	SOYMON019	g497899	BLASTN	430	1e-27	88
2410	16	701015858H1	SOYMON038	g609224	BLASTN	431	1e-27	86
2411	16	700742849H1	SOYMON012	g497899	BLASTN	434	1e-27	88
2412	16	701102648H1	SOYMON028	g16508	BLASTN	436	1e-27	89
2413	16	700940955H1	SOYMON024	g609556	BLASTN	438	1e-27	85
2414	16	700889805H1	SOYMON024	g16508	BLASTN	441	1e-27	89
2415	16	701061930H1	SOYMON033	g16508	BLASTN	315	1e-26	77
2416	16	700901375H1	SOYMON027	g609224	BLASTN	323	1e-26	84
2417	16	700989056H1	SOYMON011	g609224	BLASTN	345	1e-26	82
2418	16	700665988H1	SOYMON005	g960356	BLASTN	420	1e-26	79
2419	16	700893352H1	SOYMON024	g497899	BLASTN	428	1e-26	87
2420	16	701122945H1	SOYMON037	g16508	BLASTN	269	1e-25	76
2421	16	700992929H1	SOYMON011	g16508	BLASTN	328	1e-25	78
2422	16	700557564H1	SOYMON001	g609224	BLASTN	413	1e-25	85
2423	16	701102620H1	SOYMON028	g609224	BLASTN	414	1e-25	82
2424	16	701062321H1	SOYMON033	g497899	BLASTN	267	1e-24	87
2425	16	701062258H1	SOYMON033	g450548	BLASTN	391	1e-24	83
2426	16	701145703H1	SOYMON031	g609224	BLASTN	411	1e-24	85
2427	16	701144943H1	SOYMON031	g450548	BLASTN	421	1e-24	85
2428	16	701134190H1	SOYMON038	g2305013	BLASTN	268	1e-23	87
2429	16	701132116H1	SOYMON038	g450548	BLASTN	385	1e-23	85
2430	16	701144522H1	SOYMON031	g450548	BLASTN	406	1e-23	85
2431	16	700738002H1	SOYMON012	g1655578	BLASTX	205	1e-21	84
2432	16	700756686H1	SOYMON014	g16508				

2442	16	700674836H1	SOYMON007	g16508	BLASTN	331	1e-17	84
2443	16	700908822H1	SOYMON022	g169665	BLASTX	171	1e-16	100
2444	16	700898670H1	SOYMON027	g166872	BLASTX	172	1e-16	93
2445	16	700650967H1	SOYMON003	g16845	BLASTX	147	1e-15	100
2446	16	701132185H1	SOYMON038	g16845	BLASTX	151	1e-15	100
2447	16	700648929H1	SOYMON003	g1033190	BLASTX	156	1e-14	96
2448	16	700960809H1	SOYMON022	g16508	BLASTN	168	1e-14	88
2449	16	701101753H1	SOYMON028	g16845	BLASTX	141	1e-13	88
2450	16	700742010H1	SOYMON012	g609224	BLASTN	273	1e-13	88
2451	16	700735080H1	SOYMON010	g166874	BLASTX	121	1e-12	86
2452	16	700979242H1	SOYMON009	g609224	BLASTN	165	1e-12	81
2453	16	700740838H1	SOYMON012	g609224	BLASTN	253	1e-12	88
2454	16	700832383H1	SOYMON019	g16845	BLASTX	82	1e-11	71
2455	16	700830938H1	SOYMON019	g16845	BLASTX	92	1e-11	90
2456	16	700564686H1	SOYMON002	g1655578	BLASTX	95	1e-11	97
2457	16	700753178H1	SOYMON014	g16845	BLASTX	107	1e-10	78
2458	16	700563834H1	SOYMON002	g497900	BLASTX	124	1e-10	100
2459	16	700897739H1	SOYMON027	g16845	BLASTX	125	1e-10	86
2460	16	700725722H1	SOYMON009	g609225	BLASTX	125	1e-10	92
2461	16	701210357H1	SOYMON035	g497900	BLASTX	128	1e-10	100
2462	16	700833654H1	SOYMON019	g609224	BLASTN	243	1e-10	87
2463	16	701010334H1	SOYMON019	g609224	BLASTN	258	1e-10	86
2464	16	700567622H1	SOYMON002	g166874	BLASTX	93	1e-9	100
2465	16	700647933H1	SOYMON003	g609225	BLASTX	120	1e-9	81
2466	16	700742255H1	SOYMON012	g429107	BLASTN	160	1e-9	86
2467	16	700981506H1	SOYMON009	g609224	BLASTN	248	1e-9	88
2468	16	700962044H1	SOYMON022	g497900	BLASTX	113	1e-8	100
2469	16	701039604H1	SOYMON029	g497900	BLASTX	113	1e-8	100
2470	16	701009941H2	SOYMON019	g609225	BLASTX	114	1e-8	70
2471	16	700976956H1	SOYMON009	g609225	BLASTX	118	1e-8	96
2472	18138	701120722H1	SOYMON037	g169664	BLASTN	514	1e-33	92
2473	18138	700946044H1	SOYMON024	g169664	BLASTN	437	1e-26	90
2474	18138	700664443H1	SOYMON005	g17262	BLASTX	162	1e-16	88
2475	18138	700665162H1	SOYMON005	g17262	BLASTX	162	1e-16	88
2476	18138	701143667H1	SOYMON038	g16961	BLASTX	123	1e-11	95
2477	18138	701099150H1	SOYMON028	g16961	BLASTX	112	1e-9	90
2478	27686	700909141H1	SOYMON022	g726027	BLASTN	723	1e-51	84
2479	27686	701145458H1	SOYMON031	g726027	BLASTN	694	1e-49	86

# ADENOSYLMETHIONINE DECARBOXYLASE ( EC 4.1.1.50 )

Seq No.	Cluster ID	CloneID	Library	NCBI gi	Method	Score	P-value	%Ident
430	-700151703	700151703H1	SATMON007	g1532072	BLASTN	717	1e-50	91
431	-700165608	700165608H1	SATMON013	g1532072	BLASTN	605	1e-41	75
432	-700166868	700166868H1	SATMON013	g1532072	BLASTN	593	1e-40	90
433	-700242148	700242148H1	SATMON010	g1532048	BLASTX	142	1e-12	88
434	-700354923	700354923H1	SATMON024	g1532072	BLASTN	887	1e-74	89
435	-700422279	700422279H1	SATMONN01	g1532072	BLASTN	623	1e-61	88
436	-700455682	700455682H1	SATMON029	g1532047	BLASTN	681	1e-47	77
437	-700477645	700477645H1	SATMON025	g1532048	BLASTX	90	1e-12	71
438	-700550509	700550509H1	SATMON022	g1532047	BLASTN	399	1e-41	80
439	-700572502	700572502H1	SATMON030	g1532048	BLASTX	93	1e-20	70
440	-700618258	700618258H1	SATMON033	g1532072	BLASTN	368	1e-67	92

441	-701165456	701165456H1	SATMONN04	g1532072	BLASTN	436	1e-26	71
442	-L30622289	LIB3062-004-Q1-K1-E10	LIB3062	g1532047	BLASTN	406	1e-26	82
443	-L30623185	LIB3062-026-Q1-K1-E2	LIB3062	g1532072	BLASTN	338	1e-19	62
444	-L30625601	LIB3062-033-Q1-K1-A9	LIB3062	g1403043	BLASTN	758	1e-54	68
445	-L30661842	LIB3066-009-Q1-K1-E4	LIB3066	g1532072	BLASTN	398	1e-22	86
446	-L30681932	LIB3068-020-Q1-K1-C8	LIB3068	g1532072	BLASTN	205	1e-28	89
447	-L30687176	LIB3068-059-Q1-K1-C1	LIB3068	g1532072	BLASTN	216	1e-20	87
448	-L30692075	LIB3069-004-Q1-K1-G11	LIB3069	g1532072	BLASTN	619	1e-40	90
449	-L30783194	LIB3078-052-Q1-K1-E4	LIB3078	g1532072	BLASTN	447	1e-40	76
450	-L30792336	LIB3079-021-Q1-K1-E12	LIB3079	g1532072	BLASTN	217	1e-13	80
451	1471	700106762H1	SATMON010	g1532072	BLASTN	323	1e-59	84
452	1471	701163744H1	SATMONN04	g1532072	BLASTN	220	1e-41	86
453	1471	LIB3059-023-Q1-K1-A11	LIB3059	g1532072	BLASTN	340	1e-39	80
454	1471	700574530H1	SATMON030	g1532072	BLASTN	247	1e-34	82
455	1471	LIB83-003-Q1-E1-G1	LIB83	g1532072	BLASTN	239	1e-24	80
456	1471	701161147H1	SATMONN04	g1532072	BLASTN	239	1e-16	80
457	1471	700477336H1	SATMON025	g1532072	BLASTN	235	1e-10	91
458	16729	700169502H1	SATMON013	g1403043	BLASTN	456	1e-28	65
459	16729	700088201H1	SATMON011	g1532048	BLASTX	123	1e-22	60
460	16729	700165440H1	SATMON013	g1532048	BLASTX	162	1e-15	65
461	16866	700072905H1	SATMON007	g1532047	BLASTN	368	1e-19	71
462	16866	700350558H1	SATMON023	g1532047	BLASTN	301	1e-14	70
463	16866	700088370H1	SATMON011	g1532047	BLASTN	306	1e-14	70
464	2324	LIB3066-042-Q1-K1-H2	LIB3066	g1403043	BLASTN	1344	1e-103	79
465	2324	LIB3059-011-Q1-K1-B2	LIB3059	g1403043	BLASTN	1351	1e-103	78
466	2324	LIB3059-042-Q1-K1-B7	LIB3059	g1403043	BLASTN	1230	1e-100	80
467	2324	LIB3062-036-Q1-K1-F2	LIB3062	g1532072	BLASTN	1097	1e-91	80
468	2324	LIB3069-020-Q1-K1-A5	LIB3069	g1403043	BLASTN	925	1e-82	80
469	2324	LIB189-023-Q1-E1-H3	LIB189	g1532072	BLASTN	1085	1e-81	78
470	2324	700266088H1	SATMON017	g1532047	BLASTN	998	1e-74	80
471	2324	LIB3062-026-Q1-K1-E6	LIB3062	g1532047	BLASTN	982	1e-72	77
472	2324	700083335H1	SATMON011	g1403043	BLASTN	960	1e-71	79
473	2324	LIB189-015-Q1-E1-D3	LIB189	g1532047	BLASTN	970	1e-71	77
474	2324	LIB3079-013-Q1-K1-B3	LIB3079	g1403043	BLASTN	953	1e-70	81
475	2324	700262129H1	SATMON017	g1403043	BLASTN	936	1e-69	79
476	2324	700265667H1	SATMON017	g1403043	BLASTN	694	1e-66	81
477	2324	700807255H1	SATMON036	g1532072	BLASTN	850	1e-62	81
478	2324	700196129H1	SATMON014	g1403043	BLASTN	853	1e-62	83
479	2324	LIB3066-042-Q1-K1-H1	LIB3066	g1403043	BLASTN	842	1e-61	80
480	2324	700458321H1	SATMON029	g1403043	BLASTN	501	1e-58	80
481	2324	700197643H1	SATMON014	g1403043	BLASTN	461	1e-57	80
482	2324	LIB143-029-Q1-E1-H3	LIB143	g1532072	BLASTN	786	1e-56	78
483	2324	700197842H1	SATMON014	g1403043	BLASTN	688	1e-48	85
484	2324	700196807H1	SATMON014	g1532072	BLASTN	678	1e-47	75
485	2324	700263712H1	SATMON017	g1532072	BLASTN	403	1e-45	75
486	2324	LIB143-006-Q1-E1-F9	LIB143	g1403043	BLASTN	324	1e-42	83
487	2324	700267496H1	SATMON017	g1532047	BLASTN	594	1e-40	83
488	2324	700172551H1	SATMON013	g1532072	BLASTN	546	1e-36	74
489	2324	700211893H1	SATMON016	g1532047	BLASTN	542	1e-35	82
490	2324	700264065H1	SATMON017	g1532047	BLASTN	515	1e-34	83
491	2324	700465305H1	SATMON025	g1532073	BLASTX	148	1e-26	61
492	2324	700455052H1	SATMON029	g1403043	BLASTN	274	1e-26	79
493	2324	700473305H1	SATMON025	g1403043	BLASTN	446	1e-26	74
494	2324	700475851H1	SATMON025	g1532047	BLASTN	333	1e-18	78

495	2324	700263111H1	SATMON017	g1532073	BLASTX	165	1e-15	72
496	2324	700465705H1	SATMON025	g1403044	BLASTX	78	1e-8	76
497	3185	700264424H1	SATMON017	g1403043	BLASTN	469	1e-41	81
498	3185	LIB143-007-Q1-E1-H2	LIB143	g1403043	BLASTN	464	1e-36	80
499	3185	700262548H1	SATMON017	g1532047	BLASTN	368	1e-34	80
500	3185	700263845H1	SATMON017	g1403043	BLASTN	455	1e-34	81
501	3185	LIB3068-025-Q1-K1-G10	LIB3068	g1403043	BLASTN	288	1e-32	81
502	3185	700264569H1	SATMON017	g1403043	BLASTN	469	1e-32	82
503	3185	700243571H1	SATMON010	g1532047	BLASTN	461	1e-28	77
504	3185	700265453H1	SATMON017	g1532047	BLASTN	269	1e-27	84
505	3185	700267779H1	SATMON017	g1532047	BLASTN	257	1e-26	85
506	3185	700382373H1	SATMON024	g1532047	BLASTN	255	1e-24	86
507	3185	700258727H1	SATMON017	g1532047	BLASTN	255	1e-24	84
508	3185	LIB3069-018-Q1-K1-F5	LIB3069	g1532047	BLASTN	255	1e-21	77
509	3185	LIB3066-038-Q1-K1-C2	LIB3066	g1403043	BLASTN	242	1e-17	68
510	3185	700334654H1	SATMON019	g1532047	BLASTN	248	1e-17	90
511	3185	700262830H1	SATMON017	g1403043	BLASTN	276	1e-12	78
512	3185	700264727H1	SATMON017	g1532047	BLASTN	255	1e-10	92
513	3185	700238407H1	SATMON010	g1532047	BLASTN	255	1e-10	92
514	3185	700441952H1	SATMON026	g1532047	BLASTN	255	1e-10	92
515	3185	700268188H1	SATMON017	g1532047	BLASTN	255	1e-10	92
516	3185	700801627H1	SATMON036	g1532047	BLASTN	255	1e-10	92
517	3185	700261609H1	SATMON017	g1532047	BLASTN	255	1e-10	92
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519	3185	700258779H1	SATMON017	g1532047	BLASTN	255	1e-10	92
520	3185	700256838H1	SATMON017	g1532047	BLASTN	255	1e-10	92
521	3185	700263361H1	SATMON017	g1532047	BLASTN	255	1e-10	92
522	3185	700257102H1	SATMON017	g1532047	BLASTN	255	1e-10	92
523	3185	700239452H1	SATMON010	g1532047	BLASTN	245	1e-9	91
524	3185	700262045H1	SATMON017	g1532047	BLASTN	250	1e-9	90
525	8	LIB3066-027-Q1-K1-C6	LIB3066	g1532072	BLASTN	1414	1e-181	97
526	8	LIB3066-048-Q1-K1-C9	LIB3066	g1532072	BLASTN	1715	1e-173	98
527	8	LIB3066-007-Q1-K1-B4	LIB3066	g1532072	BLASTN	2146	1e-170	97
528	8	LIB3066-019-Q1-K1-B9	LIB3066	g1532072	BLASTN	1884	1e-168	98
529	8	LIB148-038-Q1-E1-A3	LIB148	g1532072	BLASTN	1585	1e-164	99
530	8	LIB3068-002-Q1-K1-H5	LIB3068	g1532072	BLASTN	2051	1e-162	98
531	8	LIB3067-035-Q1-K1-E9	LIB3067	g1532072	BLASTN	1594	1e-161	98
532	8	LIB189-020-Q1-E1-E8	LIB189	g1532072	BLASTN	1501	1e-160	98
533	8	LIB3078-057-Q1-K1-C12	LIB3078	g1532072	BLASTN	2026	1e-160	96
534	8	LIB3066-053-Q1-K1-A8	LIB3066	g1532072	BLASTN	1713	1e-159	93
535	8	LIB189-006-Q1-E1-C6	LIB189	g1532072	BLASTN	1676	1e-157	98
536	8	LIB3078-054-Q1-K1-E4	LIB3078	g1532072	BLASTN	1997	1e-157	95
537	8	LIB3069-028-Q1-K1-A11	LIB3069	g1532072	BLASTN	1980	1e-156	96
538	8	LIB3069-045-Q1-K1-H7	LIB3069	g1532072	BLASTN	1989	1e-156	98
539	8	LIB148-025-Q1-E1-F7	LIB148	g1532072	BLASTN	1954	1e-154	97
540	8	LIB189-014-Q1-E1-F11	LIB189	g1532072	BLASTN	1925	1e-151	94
541	8	LIB3060-014-Q1-K1-C5	LIB3060	g1532072	BLASTN	1780	1e-150	96
542	8	LIB148-057-Q1-E1-C2	LIB148	g1532072	BLASTN	1000	1e-149	98
543	8	LIB148-015-Q1-E1-F2	LIB148	g1532072	BLASTN	1886	1e-148	94
544	8	LIB3066-045-Q1-K1-A2	LIB3066	g1532072	BLASTN	914	1e-146	91
545	8	LIB148-064-Q1-E1-A3	LIB148	g1532072	BLASTN	1660	1e-146	93
546	8	LIB3061-047-Q1-K1-G1	LIB3061	g1532072	BLASTN	1867	1e-146	92
547	8	LIB148-040-Q1-E1-F1	LIB148	g1532072	BLASTN	1706	1e-145	94
548	8	LIB3069-012-Q1-K1-B6	LIB3069	g1532072	BLASTN	1630	1e-144	87

549	8	LIB3068-009-Q1-K1-A8	LIB3068	g1532072	BLASTN	1802	1e-141	97
550	8	LIB148-004-Q1-E1-D2	LIB148	g1532072	BLASTN	818	1e-139	91
551	8	LIB3068-002-Q1-K1-A1	LIB3068	g1532072	BLASTN	944	1e-137	95
552	8	LIB3067-035-Q1-K1-H9	LIB3067	g1532072	BLASTN	1701	1e-137	98
553	8	LIB3068-033-Q1-K1-G12	LIB3068	g1532072	BLASTN	1093	1e-130	89
554	8	700572229H1	SATMON030	g1532072	BLASTN	1135	1e-128	99
555	8	LIB3078-052-Q1-K1-E1	LIB3078	g1532072	BLASTN	1235	1e-127	85
556	8	700572579H1	SATMON030	g1532072	BLASTN	1625	1e-126	98
557	8	LIB3066-025-Q1-K1-F2	LIB3066	g1532072	BLASTN	1625	1e-126	100
558	8	LIB3068-048-Q1-K1-F9	LIB3068	g1532072	BLASTN	1538	1e-125	98
559	8	700098413H1	SATMON009	g1532072	BLASTN	1595	1e-124	100
560	8	700573235H1	SATMON030	g1532072	BLASTN	1513	1e-123	98
561	8	700090946H1	SATMON011	g1532072	BLASTN	1585	1e-123	100
562	8	700092465H1	SATMON008	g1532072	BLASTN	1541	1e-122	98
563	8	700074625H1	SATMON007	g1532072	BLASTN	1570	1e-122	100
564	8	LIB3059-007-Q1-K1-C10	LIB3059	g1532072	BLASTN	1401	1e-119	94
565	8	700072828H1	SATMON007	g1532072	BLASTN	1540	1e-119	100
566	8	700619106H1	SATMON034	g1532072	BLASTN	833	1e-118	97
567	8	700074853H1	SATMON007	g1532072	BLASTN	1515	1e-117	100
568	8	700201293H1	SATMON003	g1532072	BLASTN	1517	1e-117	97
569	8	700075896H1	SATMON007	g1532072	BLASTN	1006	1e-115	99
570	8	LIB3059-052-Q1-K1-A1	LIB3059	g1532072	BLASTN	1241	1e-115	92
571	8	700091576H1	SATMON011	g1532072	BLASTN	943	1e-114	98
572	8	LIB3078-015-Q1-K1-D7	LIB3078	g1532072	BLASTN	1218	1e-114	87
573	8	700074733H1	SATMON007	g1532072	BLASTN	1475	1e-114	100
574	8	700381421H1	SATMON023	g1532072	BLASTN	1475	1e-114	100
575	8	700085594H1	SATMON011	g1532072	BLASTN	1477	1e-114	99
576	8	700095883H1	SATMON008	g1532072	BLASTN	1477	1e-114	99
577	8	700338237H1	SATMON020	g1532072	BLASTN	1478	1e-114	99
578	8	700549813H1	SATMON022	g1532072	BLASTN	1481	1e-114	99
579	8	700097935H1	SATMON009	g1532072	BLASTN	1484	1e-114	98
580	8	700572978H1	SATMON030	g1532072	BLASTN	865	1e-113	96
581	8	700196464H1	SATMON014	g1532072	BLASTN	1044	1e-113	92
582	8	700381412H1	SATMON023	g1532072	BLASTN	1168	1e-113	98
583	8	700027839H1	SATMON003	g1532072	BLASTN	1472	1e-113	99
584	8	700623344H1	SATMON034	g1532072	BLASTN	1085	1e-112	94
585	8	700025858H1	SATMON003	g1532072	BLASTN	1455	1e-112	100
586	8	LIB3059-017-Q1-K1-H5	LIB3059	g1532072	BLASTN	1459	1e-112	97
587	8	700475019H1	SATMON025	g1532072	BLASTN	1390	1e-111	100
588	8	700338357H1	SATMON020	g1532072	BLASTN	1440	1e-111	100
589	8	700256796H1	SATMON017	g1532072	BLASTN	1400	1e-110	100
590	8	700071692H1	SATMON007	g1532072	BLASTN	1430	1e-110	98
591	8	700339073H1	SATMON020	g1532072	BLASTN	1357	1e-109	95
592	8	700106916H1	SATMON010	g1532072	BLASTN	1415	1e-109	93
593	8	700468234H1	SATMON025	g1532072	BLASTN	1420	1e-109	100
594	8	700214482H1	SATMON016	g1532072	BLASTN	1425	1e-109	100
595	8	700466437H1	SATMON025	g1532072	BLASTN	1403	1e-108	98
596	8	700205576H1	SATMON003	g1532072	BLASTN	1404	1e-108	98
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598	8	700348439H1	SATMON023	g1532072	BLASTN	1407	1e-108	99
599	8	700093131H1	SATMON008	g1532072	BLASTN	755	1e-107	100
600	8	700571851H1	SATMON030	g1532072	BLASTN	1302	1e-107	99
601	8	700088142H1	SATMON011	g1532072	BLASTN	1392	1e-107	99
602	8	700085934H1	SATMON011	g1532072	BLASTN	1397	1e-107	98



603	8	700028465H1	SATMON003	g1532072	BLASTN	1258	1e-106	98
604	8	700236818H1	SATMON010	g1532072	BLASTN	1380	1e-106	100
605	8	700583691H1	SATMON031	g1532072	BLASTN	1382	1e-106	99
606	8	700095585H1	SATMON008	g1532072	BLASTN	1383	1e-106	94
607	8	700105140H1	SATMON010	g1532072	BLASTN	1375	1e-105	100
608	8	700338486H1	SATMON020	g1532072	BLASTN	893	1e-104	98
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611	8	700576189H1	SATMON030	g1532072	BLASTN	1254	1e-103	93
612	8	700475734H1	SATMON025	g1532072	BLASTN	1256	1e-103	99
613	8	700028218H1	SATMON003	g1532072	BLASTN	1275	1e-103	100
614	8	700088644H1	SATMON011	g1532072	BLASTN	1351	1e-103	98
615	8	700378768H1	SATMON020	g1532072	BLASTN	1067	1e-102	98
616	8	LIB3067-035-Q1-K1-H10	LIB3067	g1532072	BLASTN	1233	1e-102	95
617	8	700043466H1	SATMON004	g1532072	BLASTN	1331	1e-102	97
618	8	LIB148-057-Q1-E1-C3	LIB148	g1532072	BLASTN	1283	1e-101	92
619	8	700217574H1	SATMON016	g1532072	BLASTN	1320	1e-101	100
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621	8	700440926H1	SATMON026	g1532072	BLASTN	1323	1e-101	99
622	8	700552284H1	SATMON022	g1532072	BLASTN	1329	1e-101	97
623	8	700216613H1	SATMON016	g1532072	BLASTN	689	1e-100	97
624	8	LIB3067-054-Q1-K1-F6	LIB3067	g1532072	BLASTN	1030	1e-100	94
625	8	LIB3060-038-Q1-K1-B9	LIB3060	g1532072	BLASTN	1108	1e-100	92
626	8	700218755H1	SATMON011	g1532072	BLASTN	1116	1e-100	99
627	8	700338042H1	SATMON020	g1532072	BLASTN	1270	1e-100	99
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629	8	700578491H1	SATMON031	g1532072	BLASTN	1309	1e-100	97
630	8	700030037H1	SATMON003	g1532072	BLASTN	1310	1e-100	97
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632	8	700157357H1	SATMON012	g1532072	BLASTN	1315	1e-100	98
633	8	700476926H1	SATMON025	g1532072	BLASTN	680	1e-99	97
634	8	700475068H1	SATMON025	g1532072	BLASTN	792	1e-99	98
635	8	700469741H1	SATMON025	g1532072	BLASTN	831	1e-99	99
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638	8	700339433H1	SATMON020	g1532072	BLASTN	1295	1e-99	100
639	8	700196627H1	SATMON014	g1532072	BLASTN	1295	1e-99	100
640	8	700259354H1	SATMON017	g1532072	BLASTN	1305	1e-99	92
641	8	700610842H1	SATMON022	g1532072	BLASTN	843	1e-98	97
642	8	700218059H1	SATMON016	g1532072	BLASTN	1036	1e-98	99
643	8	700421727H1	SATMONN01	g1532072	BLASTN	1172	1e-98	97
644	8	700158660H1	SATMON012	g1532072	BLASTN	1285	1e-98	100
645	8	700806856H1	SATMON036	g1532072	BLASTN	1194	1e-97	97
646	8	700583512H1	SATMON031	g1532072	BLASTN	1219	1e-97	97
647	8	700025502H1	SATMON004					

657	8	700156129H2	SATMON007	g1532072	BLASTN	1250	1e-95	100
658	8	700203246H1	SATMON003	g1532072	BLASTN	1250	1e-95	100
659	8	700168339H1	SATMON013	g1532072	BLASTN	915	1e-94	98
660	8	LIB3067-036-Q1-K1-F9	LIB3067	g1532072	BLASTN	1110	1e-94	95
661	8	700193769H1	SATMON014	g1532072	BLASTN	1240	1e-94	100
662	8	700094014H1	SATMON008	g1532072	BLASTN	1241	1e-94	91
663	8	700020311H1	SATMON001	g1532072	BLASTN	1228	1e-93	99
664	8	700195083H1	SATMON014	g1532072	BLASTN	1229	1e-93	98
665	8	700193901H1	SATMON014	g1532072	BLASTN	1229	1e-93	98
666	8	700194639H1	SATMON014	g1532072	BLASTN	1231	1e-93	99
667	8	700350117H1	SATMON023	g1532072	BLASTN	1143	1e-92	99
668	8	700239867H1	SATMON010	g1532072	BLASTN	1210	1e-92	98
669	8	LIB3069-028-Q1-K1-D1	LIB3069	g1532072	BLASTN	1217	1e-92	94
670	8	700355756H1	SATMON024	g1532072	BLASTN	618	1e-91	96
671	8	700265492H1	SATMON017	g1532072	BLASTN	620	1e-91	94
672	8	700579021H1	SATMON031	g1532072	BLASTN	656	1e-91	96
673	8	LIB3079-015-Q1-K1-B11	LIB3079	g1532072	BLASTN	1008	1e-91	83
674	8	700572888H2	SATMON030	g1532072	BLASTN	1159	1e-91	99
675	8	701183990H1	SATMONN06	g1532072	BLASTN	1198	1e-91	93
676	8	700085757H1	SATMON011	g1532072	BLASTN	1200	1e-91	100
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678	8	700193076H1	SATMON014	g1532072	BLASTN	1208	1e-91	99
679	8	700224378H1	SATMON011	g1532072	BLASTN	696	1e-90	99
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681	8	700159338H1	SATMON012	g1532072	BLASTN	1193	1e-90	97
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683	8	700551548H1	SATMON022	g1532072	BLASTN	839	1e-89	96
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685	8	700469834H1	SATMON025	g1532072	BLASTN	650	1e-88	97
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687	8	700194845H1	SATMON014	g1532072	BLASTN	1171	1e-88	99
688	8	700445861H1	SATMON027	g1532072	BLASTN	536	1e-87	99
689	8	700100536H1	SATMON009	g1532072	BLASTN	666	1e-87	94
690	8	701163801H1	SATMONN04	g1532072	BLASTN	805	1e-87	94
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692	8	700170872H1	SATMON013	g1532072	BLASTN	943	1e-87	97
693	8	700241270H1	SATMON010	g1532072	BLASTN	1066	1e-86	96
694	8	700457981H1	SATMON029	g1532072	BLASTN	1140	1e-86	92
695	8	700158339H1	SATMON012	g1532072	BLASTN	1142	1e-86	99
696	8	700019442H1	SATMON001	g1532072	BLASTN	1145	1e-86	100
697	8	700149885H1	SATMON007	g1532072	BLASTN	1133	1e-85	99
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699	8	700244026H1	SATMON010	g1532072	BLASTN	1044	1e-84	89
700	8	LIB3060-027-Q1-K1-B2	LIB3060	g1532072	BLASTN	1117	1e-84	99
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702	8	700267487H1	SATMON017	g1532072	BLASTN	1061	1e-83	92
703	8	700152754H1	SATMON007	g1532072	BLASTN	1107	1e-83	99
704	8	700378260H1	SATMON019	g1532072	BLASTN	1112	1e-83	92
705	8	700455089H1	SATMON029	g1532072	BLASTN	583	1e-82	97
706	8	700167322H1	SATMON013	g1532072	BLASTN	1080	1e-81	98
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708	8	700204067H1	SATMON003	g1532072	BLASTN	1011	1e-80	98
709	8	700049271H1	SATMON003	g1532072	BLASTN	627	1e-79	93
710	8	700442065H1	SATMON026	g1532072	BLASTN	1043	1e-78	91

711	8	700244175H1	SATMON010	g1532072	BLASTN	1044	1e-78	90
712	8	700045296H1	SATMON004	g1532072	BLASTN	1041	1e-77	93
713	8	700578391H1	SATMON031	g1532072	BLASTN	624	1e-76	88
714	8	700346136H1	SATMON021	g1532072	BLASTN	751	1e-76	90
715	8	700457852H1	SATMON029	g1532072	BLASTN	777	1e-76	92
716	8	700570111H1	SATMON030	g1532072	BLASTN	814	1e-75	94
717	8	LIB3066-030-Q1-K1-A12	LIB3066	g1532072	BLASTN	922	1e-75	94
718	8	700149657H1	SATMON007	g1532072	BLASTN	1012	1e-75	92
719	8	LIB3060-043-Q1-K1-B4	LIB3060	g1532072	BLASTN	1013	1e-75	97
720	8	700156752H1	SATMON012	g1532072	BLASTN	1013	1e-75	98
721	8	700454114H1	SATMON029	g1532072	BLASTN	455	1e-74	93
722	8	LIB143-053-Q1-E1-E8	LIB143	g1532072	BLASTN	672	1e-74	98
723	8	700029323H1	SATMON003	g1532072	BLASTN	996	1e-74	99
724	8	700156381H1	SATMON007	g1532072	BLASTN	1002	1e-74	97
725	8	700159603H2	SATMON012	g1532072	BLASTN	1003	1e-74	94
726	8	LIB3060-035-Q1-K1-E5	LIB3060	g1532072	BLASTN	603	1e-72	94
727	8	LIB3069-025-Q1-K1-E12	LIB3069	g1532072	BLASTN	734	1e-72	90
728	8	700166680H1	SATMON013	g1532072	BLASTN	971	1e-72	99
729	8	700171123H1	SATMON013	g1532072	BLASTN	973	1e-72	94
730	8	LIB148-042-Q1-E1-G10	LIB148	g1532072	BLASTN	975	1e-72	100
731	8	700612951H1	SATMON033	g1532072	BLASTN	401	1e-71	96
732	8	700265213H1	SATMON017	g1532072	BLASTN	626	1e-71	92
733	8	LIB189-030-Q1-E1-D11	LIB189	g1532072	BLASTN	740	1e-70	92
734	8	700212877H1	SATMON016	g1532072	BLASTN	918	1e-70	93
735	8	700161545H1	SATMON012	g1532072	BLASTN	621	1e-69	99
736	8	700021259H1	SATMON001	g1532072	BLASTN	940	1e-69	93
737	8	LIB3079-015-Q1-K1-C7	LIB3079	g1532072	BLASTN	508	1e-68	96
738	8	LIB3066-055-Q1-K1-F11	LIB3066	g1532072	BLASTN	832	1e-68	85
739	8	700166771H1	SATMON013	g1532072	BLASTN	922	1e-68	88
740	8	LIB3066-003-Q1-K1-E6	LIB3066	g1532072	BLASTN	927	1e-68	86
741	8	700208131H1	SATMON016	g1532047	BLASTN	747	1e-66	85
742	8	700020688H1	SATMON001	g1532072	BLASTN	905	1e-66	90
743	8	700206405H1	SATMON003	g1532072	BLASTN	905	1e-66	100
744	8	LIB3069-042-Q1-K1-C5	LIB3069	g1532072	BLASTN	891	1e-65	99
745	8	700353835H1	SATMON024	g1532072	BLASTN	896	1e-65	96
746	8	700471533H1	SATMON025	g1532072	BLASTN	496	1e-63	99
747	8	700165425H1	SATMON013	g1532072	BLASTN	850	1e-62	100
748	8	LIB3069-054-Q1-K1-C4	LIB3069	g1532072	BLASTN	613	1e-60	87
749	8	700160896H1	SATMON012	g1532072	BLASTN	746	1e-60	95
750	8	LIB3069-042-Q1-K1-A11	LIB3069	g1532072	BLASTN	840	1e-60	98
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752	8	700571770H1	SATMON030	g1532072	BLASTN	820	1e-59	84
753	8	LIB143-024-Q1-E1-D2	LIB143	g1532072	BLASTN	813	1e-58	95
754	8	700453677H1	SATMON028	g1532072	BLASTN	508	1e-57	92
755	8	700193979H1	SATMON014	g1532072	BLASTN	790	1e-57	100
756	8	700467828H1	SATMON025	g1532072	BLASTN	779	1e-56	96
757	8	700150072H1	SATMON007	g1532072	BLASTN	776	1e-55	99
758	8	700802869H1	SATMON036	g1532072	BLASTN	702	1e-54	95
759	8	LIB148-051-Q1-E1-B12	LIB148	g1532072	BLASTN	421	1e-53	92
760	8	700075035H1	SATMON007	g1532072	BLASTN	506	1e-53	93
761	8	700471283H1	SATMON025	g1532072	BLASTN	742	1e-53	91
762	8	700166625H1	SATMON013	g1532072	BLASTN	748	1e-53	99
763	8	700019894H1	SATMON001	g1532072	BLASTN	643	1e-51	89
764	8	700204863H1	SATMON003	g1532072	BLASTN	721	1e-51	99

765	8	700431071H1	SATMONN01	g1532072	BLASTN	366	1e-50	94
766	8	700171582H1	SATMON013	g1532072	BLASTN	492	1e-50	99
767	8	700450579H1	SATMON028	g1532072	BLASTN	338	1e-49	97
768	8	700084149H1	SATMON011	g1532072	BLASTN	672	1e-47	98
769	8	700095070H1	SATMON008	g1532072	BLASTN	662	1e-46	98
770	8	700570827H1	SATMON030	g1532072	BLASTN	344	1e-44	83
771	8	LIB3061-057-Q1-K1-G12	LIB3061	g1532072	BLASTN	378	1e-43	75
772	8	700194918H1	SATMON014	g1532072	BLASTN	626	1e-43	89
773	8	700378894H1	SATMON020	g1532072	BLASTN	495	1e-42	97
774	8	700468029H1	SATMON025	g1532072	BLASTN	616	1e-42	98
775	8	LIB143-012-Q1-E1-H8	LIB143	g1532072	BLASTN	637	1e-42	98
776	8	LIB3060-037-Q1-K1-H4	LIB3060	g1532072	BLASTN	638	1e-42	86
777	8	700433327H1	SATMONN01	g1532072	BLASTN	365	1e-41	86
778	8	700623611H1	SATMON034	g1532072	BLASTN	316	1e-39	95
779	8	700166123H1	SATMON013	g1532072	BLASTN	585	1e-39	100
780	8	700616273H1	SATMON033	g1532072	BLASTN	534	1e-38	98
781	8	700464702H1	SATMON025	g1532072	BLASTN	566	1e-38	99
782	8	700338809H1	SATMON020	g1532072	BLASTN	550	1e-37	100
783	8	700092622H1	SATMON008	g1532072	BLASTN	561	1e-37	99
784	8	LIB3060-043-Q1-K1-A10	LIB3060	g1532072	BLASTN	352	1e-36	93
785	8	700265611H1	SATMON017	g1532072	BLASTN	548	1e-36	95
786	8	700100319H1	SATMON009	g1532072	BLASTN	552	1e-36	98
787	8	700092696H1	SATMON008	g1532072	BLASTN	552	1e-36	98
788	8	700076750H1	SATMON007	g1532072	BLASTN	526	1e-35	99
789	8	700082896H1	SATMON011	g1532072	BLASTN	526	1e-35	99
790	8	700075411H1	SATMON007	g1532072	BLASTN	319	1e-34	91
791	8	701178051H1	SATMONN05	g1532072	BLASTN	342	1e-34	94
792	8	700266358H1	SATMON017	g1532072	BLASTN	520	1e-34	95
793	8	700453981H1	SATMON029	g1532072	BLASTN	505	1e-33	96
794	8	700584238H1	SATMON031	g1532072	BLASTN	509	1e-33	91
795	8	700103871H1	SATMON010	g1532072	BLASTN	491	1e-32	99
796	8	700264460H1	SATMON017	g1532072	BLASTN	495	1e-32	95
797	8	700205122H1	SATMON003	g1532072	BLASTN	501	1e-32	99
798	8	700165768H1	SATMON013	g1532072	BLASTN	420	1e-31	98
799	8	700077179H1	SATMON007	g1532072	BLASTN	471	1e-30	98
800	8	700476654H1	SATMON025	g1532072	BLASTN	476	1e-30	98
801	8	700027374H1	SATMON003	g1532072	BLASTN	476	1e-30	98
802	8	700266994H1	SATMON017	g1532072	BLASTN	476	1e-30	98
803	8	700088193H1	SATMON011	g1532072	BLASTN	488	1e-30	97
804	8	700214511H1	SATMON016	g1532072	BLASTN	298	1e-29	93
805	8	700257186H1	SATMON017	g1532072	BLASTN	465	1e-29	95
806	8	700335814H1	SATMON019	g1532072	BLASTN	469	1e-29	93
807	8	700236166H1	SATMON010	g1532072	BLASTN	450	1e-28	95
808	8	700468243H1	SATMON025	g1532072	BLASTN	456	1e-28	98
809	8	700096327H1	SATMON008	g1532072	BLASTN	451	1e-27	98
810	8	70047113						

819	8	700267990H1	SATMON017	g1532072	BLASTN	361	1e-22	99
820	8	700456715H1	SATMON029	g1532072	BLASTN	293	1e-21	97
821	8	700454806H1	SATMON029	g1532072	BLASTN	356	1e-21	98
822	8	700466812H1	SATMON025	g1532072	BLASTN	380	1e-21	95
823	8	700046476H1	SATMON004	g1532072	BLASTN	381	1e-21	97
824	8	700207160H1	SATMON017	g1532072	BLASTN	393	1e-21	96
825	8	700383037H1	SATMON024	g1532072	BLASTN	280	1e-20	98
826	8	700549660H1	SATMON022	g1532072	BLASTN	376	1e-20	97
827	8	LIB3066-004-Q1-K1-G12	LIB3066	g1532072	BLASTN	379	1e-20	91
828	8	700801422H1	SATMON036	g1532072	BLASTN	321	1e-19	97
829	8	700045319H1	SATMON004	g1532072	BLASTN	336	1e-17	99
830	8	700046047H1	SATMON004	g1532072	BLASTN	336	1e-17	99
831	8	700264328H1	SATMON017	g1532072	BLASTN	339	1e-17	95
832	8	700083485H1	SATMON011	g1532072	BLASTN	341	1e-17	99
833	8	700461268H1	SATMON033	g1532072	BLASTN	342	1e-17	95
834	8	700053271H1	SATMON008	g1532072	BLASTN	311	1e-15	98
835	8	700215275H1	SATMON016	g1532072	BLASTN	321	1e-15	98
836	8	700623133H1	SATMON034	g1532072	BLASTN	275	1e-14	75
837	8	700454949H1	SATMON029	g1532072	BLASTN	280	1e-14	99
838	8	700440763H1	SATMON026	g1532072	BLASTN	307	1e-14	95
839	8	LIB3068-022-Q1-K1-C4	LIB3068	g1532072	BLASTN	150	1e-12	94
840	8	700798849H1	SATMON036	g1532072	BLASTN	281	1e-12	98
841	8	700333057H1	SATMON019	g1532072	BLASTN	184	1e-11	91
842	8	700265786H1	SATMON017	g1532072	BLASTN	200	1e-11	90
843	8	700193030H1	SATMON014	g1532072	BLASTN	158	1e-9	94
844	8	700262964H1	SATMON017	g1532047	BLASTN	171	1e-9	81
845	8	LIB3069-023-Q1-K1-B2	LIB3069	g1403043	BLASTN	195	1e-9	84
846	8	700474096H1	SATMON025	g1532072	BLASTN	199	1e-9	97
847	8	700028326H1	SATMON003	g1532072	BLASTN	151	1e-8	96
848	8011	700440327H1	SATMON026	g1532047	BLASTN	292	1e-14	85
849	851	LIB3059-052-Q1-K1-E6	LIB3059	g1403043	BLASTN	1033	1e-85	75
850	851	700090958H1	SATMON011	g1532072	BLASTN	1044	1e-78	80
851	851	700224605H1	SATMON011	g1532072	BLASTN	850	1e-62	80
852	851	700551562H1	SATMON022	g1403043	BLASTN	374	1e-55	79
853	851	700153939H1	SATMON007	g1403043	BLASTN	772	1e-55	80
854	851	700469231H1	SATMON025	g1532072	BLASTN	560	1e-52	76
855	851	700349854H1	SATMON023	g1403043	BLASTN	669	1e-46	81
856	851	701169324H1	SATMONN05	g1403043	BLASTN	669	1e-46	78
857	851	700088319H1	SATMON011	g1532072	BLASTN	492	1e-30	78
2480	-700998660	700998660H1	SOYMON018	g1531764	BLASTN	249	1e-29	90
2481	-GM927	LIB3028-005-Q1-B1-B4	LIB3028	g1421750	BLASTN	261	1e-10	80
2482	13379	700842514H1	SOYMON020	g1915980	BLASTN	575	1e-41	73
2483	13379	701042786H1	SOYMON029	g1421750	BLASTN	377	1e-20	80
2484	15556	701101584H1	SOYMON028	g1421750	BLASTN	526	1e-35	69
2485	15556	701099749H1	SOYMON028	g1155239	BLASTN	453	1e-27	72
2486	16	LIB3051-018-Q1-E1-A7	LIB3051	g1421750	BLASTN	1146	1e-93	81
2487	16	LIB3056-005-Q1-N1-E10	LIB3056	g1421750	BLASTN	944	1e-88	80
2488	16	LIB3051-101-Q1-K1-C7	LIB3051	g1421750	BLASTN	1135	1e-85	80
2489	16	LIB3056-001-Q1-B1-H12	LIB3056	g1421750	BLASTN	752	1e-82	81
2490	16	700661334H1	SOYMON005	g1421750	BLASTN	1092	1e-82	80
2491	16	LIB3056-012-Q1-N1-B12	LIB3056	g1421750	BLASTN	1085	1e-81	80
2492	16	LIB3055-011-Q1-N1-G2	LIB3055	g1421750	BLASTN	958	1e-78	79
2493	16	700663981H1	SOYMON005	g1421750	BLASTN	996	1e-74	82
2494	16	701109737H1	SOYMON036	g1421750	BLASTN	955	1e-70	83

2495	16	701130190H1	SOYMON037	g1421750	BLASTN	935	1e-69	81
2496	16	701003301H1	SOYMON019	g1421750	BLASTN	412	1e-68	82
2497	16	700894137H1	SOYMON024	g1421750	BLASTN	923	1e-68	84
2498	16	700980096H1	SOYMON009	g1421750	BLASTN	923	1e-68	81
2499	16	700942625H1	SOYMON024	g1421750	BLASTN	911	1e-67	80
2500	16	700829695H1	SOYMON019	g1421750	BLASTN	918	1e-67	84
2501	16	700662572H1	SOYMON005	g1421750	BLASTN	920	1e-67	84
2502	16	701127647H1	SOYMON037	g1421750	BLASTN	902	1e-66	84
2503	16	701014550H1	SOYMON019	g1421750	BLASTN	556	1e-65	81
2504	16	LIB3051-043-Q1-K1-G3	LIB3051	g1421750	BLASTN	698	1e-65	82
2505	16	700730914H1	SOYMON009	g1421750	BLASTN	897	1e-65	84
2506	16	701052455H1	SOYMON032	g1421750	BLASTN	876	1e-64	81
2507	16	LIB3051-074-Q1-K1-A7	LIB3051	g1421750	BLASTN	876	1e-64	75
2508	16	700874839H1	SOYMON018	g1421750	BLASTN	862	1e-63	82
2509	16	701005943H1	SOYMON019	g1421750	BLASTN	865	1e-63	82
2510	16	700663357H1	SOYMON005	g1421750	BLASTN	869	1e-63	82
2511	16	701042053H1	SOYMON029	g1421750	BLASTN	872	1e-63	81
2512	16	700971988H1	SOYMON005	g1421750	BLASTN	508	1e-61	82
2513	16	701010728H1	SOYMON019	g1421750	BLASTN	657	1e-61	81
2514	16	700987206H1	SOYMON009	g1421750	BLASTN	845	1e-61	83
2515	16	701126773H1	SOYMON037	g1421750	BLASTN	847	1e-61	79
2516	16	700764714H1	SOYMON023	g1421750	BLASTN	830	1e-60	81
2517	16	700974444H1	SOYMON005	g1421750	BLASTN	830	1e-60	81
2518	16	701118620H1	SOYMON037	g1421750	BLASTN	832	1e-60	75
2519	16	700729926H1	SOYMON009	g1421750	BLASTN	834	1e-60	79
2520	16	700945142H1	SOYMON024	g1421750	BLASTN	470	1e-59	84
2521	16	700867912H1	SOYMON016	g1421750	BLASTN	820	1e-59	82
2522	16	700746546H1	SOYMON013	g1421750	BLASTN	824	1e-59	78
2523	16	700873331H1	SOYMON018	g1421750	BLASTN	769	1e-58	82
2524	16	700738212H1	SOYMON012	g1421750	BLASTN	780	1e-56	83
2525	16	700830454H1	SOYMON019	g1421750	BLASTN	789	1e-56	81
2526	16	700726129H1	SOYMON009	g1421750	BLASTN	789	1e-56	81
2527	16	700996774H1	SOYMON018	g1421750	BLASTN	486	1e-55	81
2528	16	700746427H1	SOYMON013	g1421750	BLASTN	766	1e-55	77
2529	16	701123911H1	SOYMON037	g1421750	BLASTN	769	1e-55	76
2530	16	700745730H1	SOYMON013	g1421750	BLASTN	775	1e-55	76
2531	16	700900940H1	SOYMON027	g1421750	BLASTN	760	1e-54	78
2532	16	700846419H1	SOYMON021	g1421750	BLASTN	762	1e-54	78
2533	16	701203427H1	SOYMON035	g1421750	BLASTN	491	1e-53	84
2534	16	LIB3051-074-Q1-K1-F5	LIB3051	g1421750	BLASTN	650	1e-53	75
2535	16	700747646H1	SOYMON013	g1421750	BLASTN	747	1e-53	77
2536	16	701049823H1	SOYMON032	g1421750	BLASTN	751	1e-53	77
2537	16	701049188H1	SOYMON032	g1421750	BLASTN	731	1e-52	75
2538	16	700875156H1	SOYMON018	g1421750	BLASTN	737	1e-52	78
2539	16	700864540H1	SOYMON016	g1421750	BLASTN	741	1e-52	81
2540	16	700682329H2	SOYMON008	g1421750	BLASTN	719	1e-51	76
2541	16	700846215H1	SOYMON021	g1421750	BLASTN	575	1e-50	82
2542	16	701101327H1	SOYMON028	g1421750	BLASTN	592	1e-50	77
2543	16	LIB3055-011-Q1-N1-E7	LIB3055	g1421750	BLASTN	680	1e-50	80
2544	16	700848992H1	SOYMON021	g1421750	BLASTN	708	1e-50	76
2545	16	700966820H1	SOYMON028	g1421750	BLASTN	710	1e-50	75
2546	16	701051785H1	SOYMON032	g1421750	BLASTN	717	1e-50	75
2547	16	700681079H1	SOYMON008	g1917012	BLASTN	290	1e-49	77
2548	16	701138880H1	SOYMON038	g1421750	BLASTN	610	1e-49	78

2549	16	700872967H1	SOYMON018	g1421750	BLASTN	704	1e-49	75
2550	16	701119960H1	SOYMON037	g1421750	BLASTN	684	1e-48	81
2551	16	700873306H1	SOYMON018	g1421750	BLASTN	693	1e-48	83
2552	16	700751484H1	SOYMON014	g1421750	BLASTN	635	1e-47	82
2553	16	700958865H1	SOYMON022	g1421750	BLASTN	486	1e-46	82
2554	16	700872833H1	SOYMON018	g1421750	BLASTN	659	1e-46	84
2555	16	700871673H1	SOYMON018	g1421750	BLASTN	659	1e-46	84
2556	16	700872801H1	SOYMON018	g1421750	BLASTN	659	1e-46	84
2557	16	700847572H1	SOYMON021	g1421750	BLASTN	660	1e-46	75
2558	16	700728046H1	SOYMON009	g1421750	BLASTN	661	1e-46	84
2559	16	700894721H1	SOYMON024	g1421750	BLASTN	550	1e-43	84
2560	16	700730946H1	SOYMON009	g1421750	BLASTN	307	1e-42	77
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2562	16	701101238H1	SOYMON028	g1421750	BLASTN	456	1e-42	92
2563	16	700758519H1	SOYMON015	g1421750	BLASTN	358	1e-41	77
2564	16	700740343H1	SOYMON012	g1531764	BLASTN	607	1e-41	72
2565	16	700848076H1	SOYMON021	g1531764	BLASTN	469	1e-40	89
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2568	16	700873876H1	SOYMON018	g1421750	BLASTN	465	1e-37	80
2569	16	700746271H1	SOYMON013	g1421750	BLASTN	515	1e-37	80
2570	16	700662887H1	SOYMON005	g1421750	BLASTN	506	1e-35	82
2571	16	700983591H1	SOYMON009	g1421750	BLASTN	537	1e-35	82
2572	16	700752343H1	SOYMON014	g1421750	BLASTN	488	1e-34	73
2573	16	700901973H1	SOYMON027	g1421750	BLASTN	490	1e-34	82
2574	16	LIB3053-013-Q1-N1-B11	LIB3053	g1421750	BLASTN	522	1e-34	75
2575	16	700988481H1	SOYMON009	g1421750	BLASTN	506	1e-33	78
2576	16	LIB3051-078-Q1-K1-C12	LIB3051	g1421750	BLASTN	302	1e-32	75
2577	16	700985640H1	SOYMON009	g1421750	BLASTN	449	1e-30	86
2578	16	701127038H1	SOYMON037	g1421750	BLASTN	467	1e-30	67
2579	16	700898677H1	SOYMON027	g1531764	BLASTN	474	1e-30	89
2580	16	700742260H1	SOYMON012	g1421750	BLASTN	476	1e-30	70
2581	16	701103203H1	SOYMON028	g1421750	BLASTN	287	1e-27	81
2582	16	700897144H1	SOYMON027	g1421750	BLASTN	264	1e-26	75
2583	16	700832039H1	SOYMON019	g1421750	BLASTN	390	1e-26	82
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2585	16	LIB3040-044-Q1-E1-B8	LIB3040	g1421750	BLASTN	282	1e-25	92
2586	16	LIB3051-024-Q1-K1-C4	LIB3051	g2394382	BLASTX	110	1e-24	95
2587	16	701011765H1	SOYMON019	g1421750	BLASTN	383	1e-21	76
2588	16	700893428H1	SOYMON024	g1421752	BLASTX	106	1e-20	82
2589	16	700996204H1	SOYMON018	g1421750	BLASTN	288	1e-20	75
2590	16	LIB3049-028-Q1-E1-C6	LIB3049	g1421750	BLASTN	230	1e-19	90
2591	16	701103628H1	SOYMON028	g1421750	BLASTN	277	1e-18	73
2592	16	700972636H1	SOYMON005	g1421750	BLASTN	286	1e-16	73
2593	16	700875417H1	SOYMON018	g1421750	BLASTN	209	1e-15	75
2594	16	701207702H1	SOYMON035	g1421752	BLASTX	153	1e-14	83
2595	16	701054556H1	SOYMON032	g1421750	BLASTN	230	1e-14	75
2596	16	701046407H1	SOYMON032	g1421750	BLASTN	230	1e-14	75
2597	16	701117634H1	SOYMON037	g1421750	BLASTN	230	1e-14	74
2598	16	701127714H1	SOYMON037	g1421750	BLASTN	230	1e-14	75
2599	16	700663239H1	SOYMON005	g1421750	BLASTN	210	1e-13	74
2600	16	700738510H1	SOYMON012	g1421750	BLASTN	212	1e-13	72
2601	16	700943559H1	SOYMON024	g1421750	BLASTN	215	1e-13	71
2602	16	700666278H1	SOYMON005	g1421750	BLASTN	290	1e-13	93

2603	16	700663144H1	SOYMON005	g1421750	BLASTN	204	1e-12	74
2604	16	700684147H1	SOYMON008	g1421750	BLASTN	278	1e-12	89
2605	16	700953076H1	SOYMON022	g1421750	BLASTN	285	1e-12	92
2606	16	701099931H1	SOYMON028	g1421750	BLASTN	202	1e-11	72
2607	16	701058910H1	SOYMON033	g1421750	BLASTN	216	1e-11	74
2608	16	700754949H1	SOYMON014	g1421750	BLASTN	266	1e-11	89
2609	16	700562967H1	SOYMON002	g1421750	BLASTN	266	1e-11	82
2610	16	701012790H1	SOYMON019	g1421750	BLASTN	251	1e-10	89
2611	16	701101739H1	SOYMON028	g1421750	BLASTN	260	1e-10	82
2612	16	701118211H1	SOYMON037	g1490554	BLASTX	97	1e-9	47
2613	16	700844394H1	SOYMON021	g1917013	BLASTX	116	1e-9	85
2614	16	701048560H1	SOYMON032	g1421750	BLASTN	198	1e-9	79
2615	16	701062373H1	SOYMON033	g1421750	BLASTN	203	1e-9	71
2616	16	701120496H1	SOYMON037	g1421750	BLASTN	230	1e-9	82
2617	16	701049680H1	SOYMON032	g1421750	BLASTN	177	1e-8	70
2618	16	700748760H1	SOYMON013	g1421750	BLASTN	230	1e-8	91
2619	16	700889482H1	SOYMON024	g1421750	BLASTN	235	1e-8	91
2620	16	700725013H1	SOYMON009	g1421750	BLASTN	239	1e-8	85
2621	16048	LIB3028-004-Q1-B1-E2	LIB3028	g1421750	BLASTN	879	1e-64	68
2622	16048	700761667H1	SOYMON015	g1421750	BLASTN	528	1e-35	71
2623	16048	700958003H1	SOYMON022	g1421750	BLASTN	428	1e-26	78

# ASPARTATE KINASE ( EC 2.7.2.4 )

Seq No.	Cluster ID	CloneID	Library	NCBI gi	Method	Score	P-value	%Ident
858	-700018870	700018870H1	SATMON001	g500850	BLASTN	1095	1e-82	100
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860	-700086169	700086169H1	SATMON011	g500852	BLASTN	559	1e-94	92
861	-700096679	700096679H1	SATMON008	g500850	BLASTN	1131	1e-85	99
862	-700096794	700096794H1	SATMON008	g500850	BLASTN	1515	1e-117	100
863	-700106390	700106390H1	SATMON010	g2243115	BLASTN	544	1e-51	72
864	-700168286	700168286H1	SATMON013	g2243115	BLASTN	519	1e-34	72
865	-700171363	700171363H1	SATMON013	g500850	BLASTN	1085	1e-81	94
866	-700194781	700194781H1	SATMON014	g2257742	BLASTN	635	1e-44	79
867	-700213839	700213839H1	SATMON016	g500850	BLASTN	664	1e-75	94
868	-700219756	700219756H1	SATMON011	g500850	BLASTN	1359	1e-104	99
869	-700258808	700258808H1	SATMON017	g500850	BLASTN	630	1e-85	96
870	-700263439	700263439H1	SATMON017	g2243115	BLASTN	448	1e-43	76
871	-700266615	700266615H1	SATMON017	g2243116	BLASTX	179	1e-17	73
872	-700342655	700342655H1	SATMON021	g2257743	BLASTX	213	1e-22	82
873	-700343285	700343285H1	SATMON021	g2257742	BLASTN	437	1e-25	66
874	-700467533	700467533H1	SATMON025	g2243115	BLASTN	439	1e-26	79
875	-700548678	700548678H1	SATMON022	g147979	BLASTX	147	1e-13	62
876	-700613618	700613618H1	SATMON033	g500850	BLASTN	965	1e-81	98
877	-L30691987	LIB3069-018-Q1-K1-A3	LIB3069	g2243115	BLASTN	266	1e-10	57
878	12201	700457103H1	SATMON029	g2257742	BLASTN	789	1e-56	76
879	12201	700457111H1	SATMON029	g2243115	BLASTN	513	1e-33	76
880	12931	700380864H1	SATMON023	g500852	BLASTN	1450	1e-111	95
881	12931	700105610H1	SATMON010	g500852	BLASTN	1046	1e-105	95
882	12931	700380848H1	SATMON023	g500852	BLASTN	1193	1e-96	95
883	12931	700205392H1	SATMON003	g500852	BLASTN	1065	1e-94	93
884	12931	700552314H1	SATMON022	g500852	BLASTN	908	1e-86	90
885	12931	700551915H1	SATMON022	g500852	BLASTN	1090	1e-81	90



886	16037	700344509H1	SATMON021	g500852	BLASTN	1184	1e-89	92
887	16037	700345170H1	SATMON021	g500852	BLASTN	600	1e-58	85
888	16157	700212607H1	SATMON016	g2243115	BLASTN	914	1e-67	76
889	16157	700094809H1	SATMON008	g2243116	BLASTX	144	1e-12	84
890	19231	700091761H1	SATMON011	g500850	BLASTN	1358	1e-104	98
891	19231	700612568H1	SATMON033	g500850	BLASTN	1137	1e-102	99
892	22303	700553291H1	SATMON022	g2243115	BLASTN	644	1e-44	70
893	22303	700553382H1	SATMON022	g2243115	BLASTN	418	1e-39	70
894	28000	LIB143-061-Q1-E1-C7	LIB143	g2243115	BLASTN	1132	1e-85	75
895	28000	700474110H1	SATMON025	g2243115	BLASTN	509	1e-33	75
896	30401	700620948H1	SATMON034	g500852	BLASTN	327	1e-30	88
897	32907	LIB143-038-Q1-E1-B11	LIB143	g500850	BLASTN	1864	1e-146	96
898	32907	700096779H1	SATMON008	g500850	BLASTN	1490	1e-115	97
899	5616	700346488H1	SATMON021	g2243115	BLASTN	664	1e-46	71
900	5616	700196138H1	SATMON014	g2243115	BLASTN	630	1e-43	74
2624	-700556108	700556108H1	SOYMON001	g2243115	BLASTN	700	1e-49	74
2625	-700663367	700663367H1	SOYMON005	g2243115	BLASTN	737	1e-52	77
2626	-700733301	700733301H1	SOYMON010	g2243115	BLASTN	751	1e-53	78
2627	-700747979	700747979H1	SOYMON013	g2257742	BLASTN	449	1e-27	70
2628	-700832664	700832664H1	SOYMON019	g167547	BLASTN	322	1e-44	78
2629	-700843925	700843925H1	SOYMON021	g167547	BLASTN	616	1e-42	71
2630	-700888516	700888516H1	SOYMON024	g464225	BLASTX	193	1e-19	78
2631	-700892002	700892002H1	SOYMON024	g2243115	BLASTN	363	1e-21	79
2632	-700959057	700959057H1	SOYMON022	g2257742	BLASTN	497	1e-32	72
2633	-700971891	700971891H1	SOYMON005	g167547	BLASTN	699	1e-49	74
2634	-700984812	700984812H1	SOYMON009	g2257742	BLASTN	801	1e-57	77
2635	-701069254	701069254H1	SOYMON034	g2243115	BLASTN	260	1e-23	75
2636	-701120341	701120341H1	SOYMON037	g2243115	BLASTN	567	1e-38	77
2637	-GM35173	LIB3051-037-Q1-K1-B9	LIB3051	g2970554	BLASTN	193	1e-11	83
2638	15020	700557507H1	SOYMON001	g167547	BLASTN	914	1e-67	79
2639	15020	700666142H1	SOYMON005	g1107460	BLASTN	767	1e-55	77
2640	18237	700797368H1	SOYMON017	g2257742	BLASTN	819	1e-59	81
2641	18237	700797360H1	SOYMON017	g2257742	BLASTN	809	1e-58	84
2642	19332	LIB3056-004-Q1-N1-D5	LIB3056	g2243115	BLASTN	1118	1e-84	74
2643	19332	700786255H2	SOYMON011	g2257742	BLASTN	626	1e-43	72
2644	19332	700684751H1	SOYMON008	g2257742	BLASTN	583	1e-39	73
2645	21954	701100440H1	SOYMON028	g167547	BLASTN	835	1e-60	78
2646	21954	701059173H1	SOYMON033	g167547	BLASTN	719	1e-51	75
2647	26336	701003103H1	SOYMON019	g2243115	BLASTN	877	1e-64	79
2648	26336	700976874H1	SOYMON009	g2243115	BLASTN	880	1e-64	79

ASPARTATE-SEMIALDEHYDE DEHYDROGENASE ( EC 1.2.1.11 )

Seq No.	Cluster ID	CloneID	Library	NCBI gi	Method	Score	P-value	%Ident
901	-700439614	700439614H1	SATMON026	g2314350	BLASTX	107	1e-13	52
902	-701183695	701183695H1	SATMONN06	g289910	BLASTX	150	1e-13	69
903	-L1487398	LIB148-064-Q1-E1-D10	LIB148	g1749466	BLASTX	178	1e-33	55
904	-L30622830	LIB3062-028-Q1-K1-A8	LIB3062	g1085109	BLASTX	108	1e-40	48
2649	-700756763	700756763H1	SOYMON014	g1359593	BLASTX	71	1e-8	52
2650	-700830054	700830054H1	SOYMON019	g142828	BLASTX	87	1e-10	41
2651	-701105617	701105617H1	SOYMON036	g142828	BLASTX	215	1e-22	57
2652	-GM8539	LIB3039-047-Q1-E1-C6	LIB3039	g142828	BLASTX	188	1e-35	50
2653	30187	LIB3049-001-Q1-E1-F2	LIB3049	g1359593	BLASTX	136	1e-26	54

2654 30187 700556105H1 SOYMON001 g1359593 BLASTX 132 1e-11 56

**O-SUCCINYLMOMOSERINE (THIOL)-LYASE ( EC 4.2.99.9 )**

Seq No.	Cluster ID	CloneID	Library	NCBI gi	Method	Score	P-value	%Ident
905	-700049526	700049526H1	SATMON003	g2198852	BLASTN	251	1e-9	76
906	-700086788	700086788H1	SATMON011	g2198850	BLASTN	631	1e-61	88
907	-700460589	700460589H1	SATMON030	g2198850	BLASTN	251	1e-32	83
908	-700577561	700577561H1	SATMON031	g2198852	BLASTN	207	1e-15	82
909	-700579840	700579840H1	SATMON031	g2198850	BLASTN	216	1e-12	94
910	-700616013	700616013H1	SATMON033	g2198852	BLASTN	278	1e-39	81
911	-L1892785	LIB189-011-Q1-E1-A10	LIB189	g2198852	BLASTN	311	1e-14	82
912	-L30594402	LIB3059-042-Q1-K1-E11	LIB3059	g2198852	BLASTN	489	1e-40	84
913	-L30604293	LIB3060-028-Q1-K1-E7	LIB3060	g2198852	BLASTN	380	1e-20	83
914	-L30693289	LIB3069-016-Q1-K1-G10	LIB3069	g2198852	BLASTN	197	1e-10	79
915	10571	700224881H1	SATMON011	g2198852	BLASTN	319	1e-15	75
916	10801	700429049H1	SATMONN01	g2198852	BLASTN	210	1e-16	82
917	10801	700167813H1	SATMON013	g2198852	BLASTN	200	1e-15	82
918	10801	700074027H1	SATMON007	g2198852	BLASTN	178	1e-11	80
919	16379	LIB3061-035-Q1-K1-B6	LIB3061	g2198850	BLASTN	2184	1e-173	99
920	16379	700259309H1	SATMON017	g2198850	BLASTN	1259	1e-108	92
921	16379	700051696H1	SATMON003	g2198850	BLASTN	1392	1e-107	96
922	16379	700239553H1	SATMON010	g2198850	BLASTN	1265	1e-96	96
923	16379	700042846H1	SATMON004	g2198850	BLASTN	1208	1e-91	95
924	16379	700092741H1	SATMON008	g2198850	BLASTN	1042	1e-89	95
925	16379	LIB3061-017-Q1-K1-D7	LIB3061	g2198850	BLASTN	1183	1e-89	99
926	16379	700206765H1	SATMON003	g2198850	BLASTN	1095	1e-82	81
927	16379	700150281H1	SATMON007	g2198850	BLASTN	1024	1e-76	94
928	16379	700165862H1	SATMON013	g2198850	BLASTN	948	1e-75	94
929	2221	LIB3060-017-Q1-K1-B10	LIB3060	g2198850	BLASTN	1819	1e-161	99
930	2221	LIB84-006-Q1-E1-F3	LIB84	g2198852	BLASTN	1615	1e-153	97
931	2221	700575334H1	SATMON030	g2198850	BLASTN	1570	1e-124	99
932	2221	700206250H1	SATMON003	g2198850	BLASTN	1515	1e-117	100
933	2221	700095073H1	SATMON008	g2198852	BLASTN	1490	1e-115	100
934	2221	700571230H1	SATMON030	g2198850	BLASTN	1355	1e-114	93
935	2221	700157358H1	SATMON012	g2198850	BLASTN	1370	1e-105	100
936	2221	700379811H1	SATMON021	g2198850	BLASTN	1345	1e-103	93
937	2221	700041570H1	SATMON004	g2198850	BLASTN	1325	1e-101	100
938	2221	700104063H1	SATMON010	g2198850	BLASTN	1157	1e-95	90
939	2221	700378265H1	SATMON019	g2198850	BLASTN	771	1e-94	99
940	2221	700235329H1	SATMON010	g2198850	BLASTN	1234	1e-94	93
941	2221	LIB3068-057-Q1-K1-D1	LIB3068	g2198852	BLASTN	1209	1e-91	94
942	2221	700159158H1	SATMON012	g2198850	BLASTN	1174	1e-89	94
943	2221	700580854H1	SATMON031	g2198850	BLASTN	754	1e-86	92
944	2221	700623409H1	SATMON034	g2198850	BLASTN	910	1e-84	96
945	2221	701164706H1	SATMONN04	g2198852	BLASTN	577	1e-83	94
946	2221	700164719H1	SATMON013	g2198852	BLASTN	831	1e-83	99
947	2221	700158146H1	SATMON012	g2198850	BLASTN	1100	1e-82	93
948	2221	700203970H1	SATMON003	g2198852	BLASTN	996	1e-80	99
949	2221	700158313H1	SATMON012	g2198850	BLASTN	1036	1e-77	93
950	2221	700425211H1	SATMONN01	g2198852	BLASTN	485	1e-61	96
951	2221	700167764H1	SATMON013	g2198850	BLASTN	793	1e-57	93
952	23788	700102780H1	SATMON010	g2198852	BLASTN	1131	1e-104	99

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953	23788	701167458H1	SATMONN05	g2198852	BLASTN	1069	1e-90	93
2655	-700900206	700900206H1	SOYMON027	g1742961	BLASTX	215	1e-24	78
2656	-GM40351	LIB3051-114-Q1-K1-H12	LIB3051	g2198851	BLASTX	122	1e-25	96
2657	12502	701101592H1	SOYMON028	g146846	BLASTX	103	1e-18	44
2658	12502	701106834H1	SOYMON036	g146846	BLASTX	103	1e-18	44
2659	13820	LIB3055-003-Q1-N1-F12	LIB3055	g3202028	BLASTX	193	1e-35	94
2660	8119	700989656H1	SOYMON011	g1742960	BLASTN	815	1e-59	79

#### CYSTATHIONINE $\beta$ -LYASE ( EC 4.4.1.8 )

Seq No.	Cluster ID	CloneID	Library	NCBI gi	Method	Score	P-value	%Ident
954	-700155172	700155172H1	SATMON007	g704397	BLASTX	361	1e-43	78
955	-L362943	LIB36-013-Q1-E1-G10	LIB36	g704396	BLASTN	818	1e-59	75
956	19856	700240664H1	SATMON010	g704396	BLASTN	496	1e-31	69
957	19856	700572496H1	SATMON030	g704396	BLASTN	446	1e-26	68
958	22960	701170964H1	SATMONN05	g704396	BLASTN	778	1e-56	78
959	22960	LIB3061-002-Q1-K2-F9	LIB3061	g704396	BLASTN	765	1e-53	77
960	22960	701172780H2	SATMONN05	g704396	BLASTN	686	1e-48	73
961	22960	700578571H1	SATMON031	g704397	BLASTX	222	1e-23	89
962	30752	LIB3078-055-Q1-K1-C8	LIB3078	g704396	BLASTN	747	1e-51	71
963	30752	700086603H1	SATMON011	g704397	BLASTX	192	1e-18	64
2661	-701001147	701001147H1	SOYMON018	g704396	BLASTN	847	1e-61	78
2662	18602	700566066H1	SOYMON002	g704396	BLASTN	751	1e-57	79
2663	18602	700890955H1	SOYMON024	g704396	BLASTN	698	1e-49	77
2664	18602	700896865H1	SOYMON027	g704396	BLASTN	682	1e-48	77
2665	5144	LIB3050-006-Q1-E1-A9	LIB3050	g1399263	BLASTX	96	1e-31	41

#### 5-METHYLTETRAHYDROPTEROYLTRIGLUTAMATE--HOMOCYSTEINE S-METHYLTRANSFERASE ( EC 2.1.1.14 )

Seq No.	Cluster ID	CloneID	Library	NCBI gi	Method	Score	P-value	%Ident
964	-700212217	700212217H1	SATMON016	g886471	BLASTX	151	1e-16	93
965	-700333966	700333966H1	SATMON019	g886470	BLASTN	370	1e-23	66
966	-700377403	700377403H1	SATMON019	g2738247	BLASTN	305	1e-26	80
967	-700571893	700571893H1	SATMON030	g974781	BLASTN	262	1e-20	80
968	-701165656	701165656H1	SATMONN04	g2738247	BLASTN	450	1e-50	73
969	-L30622954	LIB3062-030-Q1-K1-C9	LIB3062	g886470	BLASTN	366	1e-26	81
970	-L30662004	LIB3066-026-Q1-K1-F1	LIB3066	g2738248	BLASTX	140	1e-28	77
971	-L30671450	LIB3067-001-Q1-K1-C6	LIB3067	g2738247	BLASTN	553	1e-46	69
972	-L30694410	LIB3069-057-Q1-K1-B4	LIB3069	g886470	BLASTN	447	1e-26	67
973	1	700452404H1	SATMON028	g453939	BLASTX	59	1e-17	94
974	13513	700049012H1	SATMON003	g1814402	BLASTN	1006	1e-74	81
975	13513	700086058H1	SATMON011	g1814402	BLASTN	982	1e-72	81
976	13513	700235814H1	SATMON010	g1814402	BLASTN	890	1e-65	79
977	13513	700170015H1	SATMON013	g1814402	BLASTN	578	1e-39	78
978	3835	700093161H1	SATMON008	g974781	BLASTN	858	1e-62	76
979	3835	700223321H1	SATMON011	g974781	BLASTN	836	1e-60	80
980	3835	700454003H1	SATMON029	g974781	BLASTN	823	1e-59	82
981	3835	700238925H1	SATMON010	g2738247	BLASTN	733	1e-55	74
982	3835	700075142H1	SATMON007	g2738247	BLASTN	569	1e-52	73
983	3835	700151969H1	SATMON007	g974781	BLASTN	660	1e-46	76
984	3835	700281434H2	SATMON019	g974781	BLASTN	560	1e-45	79

985	3835	700084914H1	SATMON011	g974781	BLASTN	440	1e-36	79
986	3835	700215135H1	SATMON016	g974781	BLASTN	543	1e-36	78
987	3835	700202218H1	SATMON003	g974781	BLASTN	389	1e-23	78
988	3835	700281467H2	SATMON019	g2738248	BLASTX	123	1e-12	71
989	456	LIB3059-019-Q1-K1-G6	LIB3059	g974781	BLASTN	1493	1e-115	83
990	456	LIB3068-012-Q1-K1-G7	LIB3068	g974781	BLASTN	1471	1e-113	81
991	456	LIB3061-050-Q1-K1-G10	LIB3061	g886470	BLASTN	1285	1e-98	82
992	456	LIB3067-043-Q1-K1-F9	LIB3067	g2738247	BLASTN	1287	1e-98	79
993	456	700087169H1	SATMON011	g1814402	BLASTN	1245	1e-94	86
994	456	LIB3069-030-Q1-K1-G9	LIB3069	g1814402	BLASTN	772	1e-93	80
995	456	LIB3069-019-Q1-K1-G11	LIB3069	g1814402	BLASTN	1202	1e-91	81
996	456	700202514H1	SATMON003	g1814402	BLASTN	1134	1e-89	84
997	456	LIB3062-011-Q1-K1-F2	LIB3062	g886470	BLASTN	664	1e-86	84
998	456	LIB143-015-Q1-E1-A1	LIB143	g974781	BLASTN	1143	1e-86	81
999	456	700570326H1	SATMON030	g974781	BLASTN	1089	1e-85	83
1000	456	700103727H1	SATMON010	g2738247	BLASTN	1132	1e-85	86
1001	456	700574823H1	SATMON030	g1814402	BLASTN	1135	1e-85	80
1002	456	700091666H1	SATMON011	g1814402	BLASTN	1136	1e-85	84
1003	456	LIB3069-033-Q1-K1-E9	LIB3069	g2738247	BLASTN	1115	1e-84	79
1004	456	700572634H1	SATMON030	g1814402	BLASTN	1123	1e-84	85
1005	456	700211829H1	SATMON016	g2738247	BLASTN	1096	1e-82	84
1006	456	700087109H1	SATMON011	g1814402	BLASTN	1102	1e-82	85
1007	456	LIB3059-003-Q1-K1-B1	LIB3059	g1814402	BLASTN	961	1e-81	82
1008	456	700047556H1	SATMON003	g2738247	BLASTN	976	1e-81	82
1009	456	700201925H1	SATMON003	g2738247	BLASTN	1001	1e-81	84
1010	456	700209343H1	SATMON016	g1814402	BLASTN	1081	1e-81	82
1011	456	700348416H1	SATMON023	g1814402	BLASTN	1081	1e-81	83
1012	456	700095559H1	SATMON008	g1814402	BLASTN	1085	1e-81	82
1013	456	700073472H1	SATMON007	g1814402	BLASTN	1089	1e-81	82
1014	456	LIB3062-057-Q1-K1-C2	LIB3062	g886470	BLASTN	1073	1e-80	78
1015	456	700093445H1	SATMON008	g1814402	BLASTN	1076	1e-80	84
1016	456	LIB143-026-Q1-E1-H3	LIB143	g2738247	BLASTN	648	1e-79	79
1017	456	700206240H1	SATMON003	g1814402	BLASTN	1057	1e-79	82
1018	456	700091907H1	SATMON011	g886470	BLASTN	1062	1e-79	80
1019	456	700258178H1	SATMON017	g1814402	BLASTN	1064	1e-79	84
1020	456	700086565H1	SATMON011	g1814402	BLASTN	1065	1e-79	82
1021	456	700471452H1	SATMON025	g2738247	BLASTN	1045	1e-78	85
1022	456	700243858H1	SATMON010	g1814402	BLASTN	1048	1e-78	85
1023	456	700352224H1	SATMON023	g2738247	BLASTN	1051	1e-78	84
1024	456	700084769H1	SATMON011	g1814402	BLASTN	1054	1e-78	83
1025	456	700082818H1	SATMON011	g886470	BLASTN	984	1e-77	84
1026	456	700331974H1	SATMON019	g886470	BLASTN	1031	1e-77	82
1027	456	700086203H1	SATMON011	g1814402	BLASTN	1032	1e-77	81
1028	456	700075826H1	SATMON007	g2738247	BLASTN	1038	1e-77	82
1029	456	700104555H1	SATMON010	g886470	BLASTN	1039	1e-77	79
1030	456	700074084H1	SATMON007	g2738247	BLASTN	1039	1e-77	87
1031	456	700076872H1	SATMON007	g1814402	BLASTN	700	1e-76	83
1032	456	700050704H1	SATMON003	g1814402	BLASTN	880	1e-76	82
1033	456	700030215H1	SATMON003	g974781	BLASTN	1019	1e-76	83
1034	456	700077416H1	SATMON007	g886470	BLASTN	1019	1e-76	79
1035	456	700093982H1	SATMON008	g1814402	BLASTN	1019	1e-76	82
1036	456	700048847H1	SATMON003	g2738247	BLASTN	1023	1e-76	85
1037	456	700090739H1	SATMON011	g886470	BLASTN	1026	1e-76	82
1038	456	700092904H1	SATMON008	g886470	BLASTN	1026	1e-76	82

1039	456	LIB3068-009-Q1-K1-E5	LIB3068	g1814402	BLASTN	1027	1e-76	73
1040	456	700336705H1	SATMON019	g1814402	BLASTN	747	1e-75	83
1041	456	700344872H1	SATMON021	g1814402	BLASTN	768	1e-75	85
1042	456	LIB3066-019-Q1-K1-E1	LIB3066	g886470	BLASTN	868	1e-75	81
1043	456	700086322H1	SATMON011	g2738247	BLASTN	1015	1e-75	81
1044	456	LIB3067-052-Q1-K1-B12	LIB3067	g974781	BLASTN	1033	1e-75	81
1045	456	700221395H1	SATMON011	g2738247	BLASTN	1000	1e-74	85
1046	456	700092483H1	SATMON008	g1814402	BLASTN	1001	1e-74	82
1047	456	700618708H1	SATMON034	g886470	BLASTN	1003	1e-74	82
1048	456	700074191H1	SATMON007	g2738247	BLASTN	1005	1e-74	86
1049	456	LIB143-055-Q1-E1-F10	LIB143	g1814402	BLASTN	986	1e-73	83
1050	456	700025955H1	SATMON003	g1814402	BLASTN	993	1e-73	84
1051	456	700574824H1	SATMON030	g886470	BLASTN	564	1e-72	80
1052	456	700201527H1	SATMON003	g974781	BLASTN	812	1e-72	81
1053	456	700092202H1	SATMON008	g886470	BLASTN	946	1e-72	79
1054	456	700223674H1	SATMON011	g2738247	BLASTN	971	1e-72	83
1055	456	700092638H1	SATMON008	g1814402	BLASTN	972	1e-72	84
1056	456	700090025H1	SATMON011	g974781	BLASTN	973	1e-72	81
1057	456	700217069H1	SATMON016	g2738247	BLASTN	976	1e-72	83
1058	456	700349142H1	SATMON023	g1814402	BLASTN	980	1e-72	82
1059	456	700088387H1	SATMON011	g2738247	BLASTN	980	1e-72	85
1060	456	700215102H1	SATMON016	g886470	BLASTN	980	1e-72	79
1061	456	700456708H1	SATMON029	g2738247	BLASTN	982	1e-72	82
1062	456	700210025H1	SATMON016	g1814402	BLASTN	982	1e-72	81
1063	456	700335345H1	SATMON019	g974781	BLASTN	982	1e-72	84
1064	456	700085257H1	SATMON011	g1814402	BLASTN	554	1e-71	82
1065	456	700026781H1	SATMON003	g974781	BLASTN	961	1e-71	82
1066	456	700224265H1	SATMON011	g2738247	BLASTN	962	1e-71	83
1067	456	700083282H1	SATMON011	g1814402	BLASTN	962	1e-71	83
1068	456	700381324H1	SATMON023	g1814402	BLASTN	965	1e-71	82
1069	456	700212017H1	SATMON016	g1814402	BLASTN	640	1e-70	83
1070	456	700088053H1	SATMON011	g886470	BLASTN	853	1e-70	84
1071	456	LIB3062-032-Q1-K1-C2	LIB3062	g1814402	BLASTN	883	1e-70	80
1072	456	700083030H1	SATMON011	g1814402	BLASTN	948	1e-70	81
1073	456	700613980H1	SATMON033	g1814402	BLASTN	950	1e-70	83
1074	456	700347019H1	SATMON021	g974781	BLASTN	952	1e-70	81
1075	456	700335621H1	SATMON019	g1814402	BLASTN	952	1e-70	80
1076	456	700094101H1	SATMON008	g1814402	BLASTN	953	1e-70	83
1077	456	700073128H1	SATMON007	g2738247	BLASTN	954	1e-70	78
1078	456	700076245H1	SATMON007	g1814402	BLASTN	955	1e-70	83
1079	456	700071903H1	SATMON007	g886470	BLASTN	956	1e-70	81
1080	456	700090035H1	SATMON011	g886470	BLASTN	935	1e-69	80
1081	456	700405323H1	SATMON029	g1814402	BLASTN	937	1e-69	83
1082	456	700050121H1	SATMON003	g886470	BLASTN	938	1e-69	

1093	456	700085411H1	SATMON011	g1814402	BLASTN	723	1e-68	83
1094	456	700454357H1	SATMON029	g2738247	BLASTN	926	1e-68	82
1095	456	700620976H1	SATMON034	g1814402	BLASTN	927	1e-68	81
1096	456	700241483H1	SATMON010	g1814402	BLASTN	931	1e-68	86
1097	456	700355459H1	SATMON024	g974781	BLASTN	931	1e-68	82
1098	456	700095875H1	SATMON008	g1814402	BLASTN	931	1e-68	87
1099	456	700076702H1	SATMON007	g1814402	BLASTN	934	1e-68	84
1100	456	700212538H1	SATMON016	g1814402	BLASTN	689	1e-67	85
1101	456	700086479H1	SATMON011	g1814402	BLASTN	715	1e-67	82
1102	456	700453684H1	SATMON028	g1814402	BLASTN	747	1e-67	83
1103	456	700204454H1	SATMON003	g1814402	BLASTN	797	1e-67	82
1104	456	700612577H1	SATMON033	g974781	BLASTN	911	1e-67	81
1105	456	700474018H1	SATMON025	g886470	BLASTN	917	1e-67	81
1106	456	700213602H1	SATMON016	g886470	BLASTN	917	1e-67	76
1107	456	700076911H1	SATMON007	g974781	BLASTN	917	1e-67	81
1108	456	700223313H1	SATMON011	g886470	BLASTN	919	1e-67	84
1109	456	700220692H1	SATMON011	g974781	BLASTN	920	1e-67	81
1110	456	700258038H1	SATMON017	g886470	BLASTN	920	1e-67	83
1111	456	700095862H1	SATMON008	g1814402	BLASTN	922	1e-67	86
1112	456	700213396H1	SATMON016	g886470	BLASTN	922	1e-67	83
1113	456	700354259H1	SATMON024	g974781	BLASTN	638	1e-66	81
1114	456	700471505H1	SATMON025	g886470	BLASTN	681	1e-66	83
1115	456	700202439H1	SATMON003	g1814402	BLASTN	706	1e-66	82
1116	456	700223609H1	SATMON011	g974781	BLASTN	770	1e-66	83
1117	456	700405260H1	SATMON028	g1814402	BLASTN	782	1e-66	86
1118	456	700085214H1	SATMON011	g886470	BLASTN	814	1e-66	84
1119	456	700211194H1	SATMON016	g1814402	BLASTN	902	1e-66	84
1120	456	700106641H1	SATMON010	g1814402	BLASTN	903	1e-66	82
1121	456	700405170H1	SATMON028	g974781	BLASTN	904	1e-66	79
1122	456	700090015H1	SATMON011	g886470	BLASTN	905	1e-66	80
1123	456	700623154H1	SATMON034	g886470	BLASTN	906	1e-66	81
1124	456	700458895H1	SATMON029	g886470	BLASTN	906	1e-66	83
1125	456	700094073H1	SATMON008	g886470	BLASTN	907	1e-66	78
1126	456	700158508H1	SATMON012	g2738247	BLASTN	909	1e-66	82
1127	456	700027366H1	SATMON003	g1814402	BLASTN	910	1e-66	81
1128	456	700073636H1	SATMON007	g1814402	BLASTN	615	1e-65	87
1129	456	700220207H1	SATMON011	g886470	BLASTN	785	1e-65	84
1130	456	LIB143-007-Q1-E1-A6	LIB143	g886470	BLASTN	807	1e-65	80
1131	456	700575288H1	SATMON030	g1814402	BLASTN	887	1e-65	83
1132	456	700219870H1	SATMON011	g974781	BLASTN	887	1e-65	82
1133	456	701184573H1	SATMONN06	g974781	BLASTN	888	1e-65	81
1134	456	700076946H1	SATMON007	g1814402	BLASTN	891	1e-65	86
1135	456	700214808H1	SATMON016	g2738247	BLASTN	893	1e-65	81
1136	456	700096274H1	SATMON008	g1814402	BLASTN	894	1e-65	80
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1147	456	700093168H1	SATMON008	g1814402	BLASTN	885	1e-64	86
1148	456	700224478H1	SATMON011	g886470	BLASTN	886	1e-64	81
1149	456	700261958H1	SATMON017	g1814402	BLASTN	781	1e-63	79
1150	456	700074221H1	SATMON007	g1814402	BLASTN	825	1e-63	81
1151	456	700089966H1	SATMON011	g1814402	BLASTN	863	1e-63	84
1152	456	700210422H1	SATMON016	g1814402	BLASTN	863	1e-63	81
1153	456	700160272H1	SATMON012	g1814402	BLASTN	863	1e-63	85
1154	456	700444282H1	SATMON027	g2738247	BLASTN	549	1e-62	84
1155	456	700206489H1	SATMON003	g2738247	BLASTN	597	1e-62	80
1156	456	700446452H1	SATMON027	g974781	BLASTN	711	1e-62	80
1157	456	700265473H1	SATMON017	g1814402	BLASTN	715	1e-62	79
1158	456	700473708H1	SATMON025	g1814402	BLASTN	762	1e-62	82
1159	456	LIB3069-025-Q1-K1-A7	LIB3069	g886470	BLASTN	852	1e-62	80
1160	456	700166631H1	SATMON013	g2738247	BLASTN	858	1e-62	85
1161	456	700582413H1	SATMON031	g1814402	BLASTN	861	1e-62	78
1162	456	700071904H1	SATMON007	g2738247	BLASTN	544	1e-61	77
1163	456	700622655H1	SATMON034	g1814402	BLASTN	592	1e-61	82
1164	456	700082541H1	SATMON011	g1814402	BLASTN	793	1e-61	82
1165	456	700335378H1	SATMON019	g2738247	BLASTN	841	1e-61	76
1166	456	700203585H1	SATMON003	g2738247	BLASTN	846	1e-61	86
1167	456	700053043H1	SATMON007	g2738247	BLASTN	847	1e-61	81
1168	456	700163654H1	SATMON013	g1814402	BLASTN	474	1e-60	84
1169	456	700352269H1	SATMON023	g1814402	BLASTN	479	1e-60	84
1170	456	700106187H1	SATMON010	g1814402	BLASTN	631	1e-60	81
1171	456	LIB143-007-Q1-E1-A7	LIB143	g886470	BLASTN	759	1e-60	76
1172	456	700458694H1	SATMON029	g1814402	BLASTN	827	1e-60	86
1173	456	700213509H1	SATMON016	g886470	BLASTN	834	1e-60	81
1174	456	700150191H1	SATMON007	g2738247	BLASTN	834	1e-60	82
1175	456	700157594H1	SATMON012	g1814402	BLASTN	835	1e-60	80
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1177	456	700220254H1	SATMON011	g2738247	BLASTN	687	1e-59	77
1178	456	700095485H1	SATMON008	g886470	BLASTN	819	1e-59	77
1179	456	LIB143-006-Q1-E1-B3	LIB143	g1814402	BLASTN	830	1e-59	65
1180	456	700071763H1	SATMON007	g2738247	BLASTN	565	1e-58	75
1181	456	700082259H1	SATMON011	g1814402	BLASTN	707	1e-58	84
1182	456	700020717H1	SATMON001	g886470	BLASTN	805	1e-58	81
1183	456	700041892H1	SATMON004	g1814402	BLASTN	805	1e-58	86
1184	456	700356573H1	SATMON024	g1814402	BLASTN	811	1e-58	81
1185	456	700171632H1	SATMON013	g2738247	BLASTN	811	1e-58	85
1186	456	700222516H1	SATMON011	g1814402	BLASTN	812	1e-58	87
1187	456	700466679H1	SATMON025	g1814402	BLASTN	812	1e-58	87
1188	456	700208929H1	SATMON016	g1814402	BLASTN	407	1e-57	78
1189	456	700355014H1	SATMON024	g1814402	BLASTN	592	1e-57	85
1190	456	701159834H1	SATMONN04	g1814402	BLASTN	683	1e-57	77
1191	456	700351137H1	SATMON023	g974781	BLASTN	725	1e-57	79
1192	456	700027308H1	SATMON003	g2738247	BLASTN	791	1e-57	80
1193	456	700142587H1	SATMON012	g974781	BLASTN	791	1e-57	83
1194	456	700170157H1	SATMON013	g1814402	BLASTN	791	1e-57	81
1195	456	700019438H1	SATMON001	g974781	BLASTN	798	1e-57	82
1196	456	700166430H1	SATMON013	g886470	BLASTN	799	1e-57	84
1197	456	700047369H1	SATMON003	g886470	BLASTN	799	1e-57	80
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1200	456	700204588H1	SATMON003	g886470	BLASTN	720	1e-56	82

1201	456	700071667H1	SATMON007	g1814402	BLASTN	787	1e-56	85
1202	456	701185269H1	SATMONN06	g886470	BLASTN	598	1e-55	76
1203	456	700243844H1	SATMON010	g2738247	BLASTN	769	1e-55	79
1204	456	700221969H1	SATMON011	g974781	BLASTN	771	1e-55	79
1205	456	700335706H1	SATMON019	g1814402	BLASTN	773	1e-55	80
1206	456	700622285H1	SATMON034	g1814402	BLASTN	774	1e-55	82
1207	456	700089665H1	SATMON011	g2738247	BLASTN	774	1e-55	78
1208	456	700464833H1	SATMON025	g2738247	BLASTN	455	1e-54	72
1209	456	LIB3069-057-Q1-K1-A6	LIB3069	g974781	BLASTN	564	1e-54	76
1210	456	700156818H1	SATMON012	g974781	BLASTN	757	1e-54	81
1211	456	700219420H1	SATMON011	g974781	BLASTN	758	1e-54	79
1212	456	700152570H1	SATMON007	g1814402	BLASTN	761	1e-54	86
1213	456	700084484H1	SATMON011	g1814402	BLASTN	764	1e-54	84
1214	456	700152208H1	SATMON007	g1814402	BLASTN	765	1e-54	82
1215	456	700550889H1	SATMON022	g2738247	BLASTN	546	1e-53	76
1216	456	700025869H1	SATMON003	g2738247	BLASTN	675	1e-53	79
1217	456	700383075H1	SATMON024	g974781	BLASTN	724	1e-53	81
1218	456	700575104H1	SATMON030	g1814402	BLASTN	745	1e-53	70
1219	456	LIB3069-022-Q1-K1-C1	LIB3069	g974781	BLASTN	768	1e-53	81
1220	456	700215272H1	SATMON016	g1814402	BLASTN	674	1e-52	83
1221	456	700167881H1	SATMON013	g974781	BLASTN	737	1e-52	79
1222	456	700019727H1	SATMON001	g2738247	BLASTN	737	1e-52	81
1223	456	700351776H1	SATMON023	g886470	BLASTN	741	1e-52	76
1224	456	700210543H1	SATMON016	g974781	BLASTN	742	1e-52	85
1225	456	700571604H1	SATMON030	g1814402	BLASTN	360	1e-51	76
1226	456	700169777H1	SATMON013	g1814402	BLASTN	426	1e-51	83
1227	456	700096744H1	SATMON008	g2738247	BLASTN	539	1e-51	79
1228	456	700383157H1	SATMON024	g974781	BLASTN	667	1e-51	79
1229	456	700155764H1	SATMON007	g886470	BLASTN	721	1e-51	75
1230	456	700150817H1	SATMON007	g1814402	BLASTN	727	1e-51	75
1231	456	700619660H1	SATMON034	g886470	BLASTN	727	1e-51	73
1232	456	700471085H1	SATMON025	g1814402	BLASTN	406	1e-50	79
1233	456	700163226H1	SATMON013	g2738247	BLASTN	475	1e-50	78
1234	456	700444567H1	SATMON027	g1814402	BLASTN	498	1e-50	81
1235	456	700457303H1	SATMON029	g2738247	BLASTN	662	1e-50	80
1236	456	700088065H1	SATMON011	g886470	BLASTN	707	1e-50	79
1237	456	700152592H1	SATMON007	g886470	BLASTN	716	1e-50	80
1238	456	LIB3068-061-Q1-K1-B2	LIB3068	g886470	BLASTN	730	1e-50	78
1239	456	700331880H1	SATMON019	g1814402	BLASTN	697	1e-49	85
1240	456	700218025H1	SATMON016	g886470	BLASTN	700	1e-49	79
1241	456	700348643H1	SATMON023	g1814402	BLASTN	701	1e-49	80
1242	456	700236975H1	SATMON010	g974781	BLASTN	704	1e-49	81
1243	456	700442968H1	SATMON026	g886470	BLASTN	418	1e-48	79
1244	456	700479529H1	SATMON034	g2738247	BLASTN	508	1e-48	75
1245	456	700160983H1	SATMON012	g1814402	BLASTN	690	1e-48	83
1246	456	700050185H1	SATMON003	g974781	BLASTN	421	1e-47	75
1247	456	700611764H1	SATMON022	g1814402	BLASTN	503	1e-47	79
1248	456	700622669H1	SATMON034	g1814402	BLASTN	549	1e-47	79
1249	456	700153237H1	SATMON007	g1814402	BLASTN	672	1e-47	80
1250	456	700242703H1	SATMON010	g886470	BLASTN	682	1e-47	81
1251	456	700165177H1	SATMON013	g2738247	BLASTN	682	1e-47	78
1252	456	700379889H1	SATMON021	g886470	BLASTN	682	1e-47	85
1253	456	700449243H1	SATMON028	g1814402	BLASTN	357	1e-46	83
1254	456	700453282H1	SATMON028	g886470	BLASTN	608	1e-46	81



1255	456	700224308H1	SATMON011	g1814402	BLASTN	661	1e-46	83
1256	456	700150484H1	SATMON007	g886470	BLASTN	663	1e-46	78
1257	456	700240609H1	SATMON010	g2738247	BLASTN	668	1e-46	79
1258	456	700171610H1	SATMON013	g974781	BLASTN	669	1e-46	79
1259	456	LIB143-027-Q1-E1-H3	LIB143	g1814402	BLASTN	670	1e-46	86
1260	456	700334084H1	SATMON019	g1814402	BLASTN	467	1e-45	81
1261	456	LIB3069-043-Q1-K1-H6	LIB3069	g886470	BLASTN	546	1e-45	83
1262	456	700222515H1	SATMON011	g1814402	BLASTN	648	1e-45	75
1263	456	700223749H1	SATMON011	g1814402	BLASTN	648	1e-45	75
1264	456	701180187H1	SATMONN05	g974781	BLASTN	648	1e-45	78
1265	456	700050111H1	SATMON003	g2738247	BLASTN	653	1e-45	82
1266	456	700051275H1	SATMON003	g1814402	BLASTN	379	1e-44	75
1267	456	700235202H1	SATMON010	g2738247	BLASTN	637	1e-44	80
1268	456	700257385H1	SATMON017	g1814402	BLASTN	638	1e-44	80
1269	456	LIB143-028-Q1-E1-H7	LIB143	g1814402	BLASTN	638	1e-44	80
1270	456	700576066H1	SATMON030	g886470	BLASTN	516	1e-43	76
1271	456	700455065H1	SATMON029	g886470	BLASTN	624	1e-43	79
1272	456	700074158H1	SATMON007	g1814402	BLASTN	630	1e-43	83
1273	456	700171322H1	SATMON013	g1814402	BLASTN	634	1e-43	77
1274	456	700457369H1	SATMON029	g974781	BLASTN	392	1e-42	83
1275	456	700378252H1	SATMON019	g2738247	BLASTN	462	1e-42	77
1276	456	700454626H1	SATMON029	g1814402	BLASTN	495	1e-42	85
1277	456	700208182H1	SATMON016	g2738247	BLASTN	564	1e-42	73
1278	456	700618848H1	SATMON034	g886470	BLASTN	615	1e-42	80
1279	456	700222969H1	SATMON011	g1814402	BLASTN	622	1e-42	74
1280	456	700444782H1	SATMON027	g1814402	BLASTN	337	1e-41	81
1281	456	700204404H1	SATMON003	g2738247	BLASTN	607	1e-41	80
1282	456	700347028H1	SATMON021	g1814402	BLASTN	598	1e-40	81
1283	456	700172447H1	SATMON013	g1814402	BLASTN	328	1e-39	85
1284	456	700549696H1	SATMON022	g886470	BLASTN	360	1e-39	81
1285	456	700257287H1	SATMON017	g1814402	BLASTN	365	1e-38	84
1286	456	700569630H1	SATMON030	g1814402	BLASTN	566	1e-38	78
1287	456	700207258H1	SATMON017	g1814402	BLASTN	568	1e-38	85
1288	456	700052467H1	SATMON003	g1814402	BLASTN	515	1e-37	82
1289	456	700083656H1	SATMON011	g2738247	BLASTN	552	1e-37	83
1290	456	700429279H1	SATMONN01	g2738247	BLASTN	556	1e-37	79
1291	456	700349921H1	SATMON023	g2738247	BLASTN	471	1e-36	78
1292	456	700449609H1	SATMON028	g886470	BLASTN	540	1e-36	81
1293	456	700150152H1	SATMON007	g1814402	BLASTN	543	1e-36	74
1294	456	700075675H1	SATMON007	g1814402	BLASTN	546	1e-36	81
1295	456	700267015H1	SATMON017	g886470	BLASTN	550	1e-36	75
1296	456	700465118H1	SATMON025	g1814402	BLASTN	555	1e-36	78
1297	456	700456662H1	SATMON029	g2738247	BLASTN	314	1e-34	76
1298	456	700405315H1	SATMON029	g886				

1309	456	700152817H1	SATMON007	g974781	BLASTN	459	1e-29	88
1310	456	700208732H1	SATMON016	g974781	BLASTN	485	1e-29	76
1311	456	700051461H1	SATMON003	g886470	BLASTN	467	1e-28	73
1312	456	700616589H1	SATMON033	g2738247	BLASTN	273	1e-27	78
1313	456	700236150H1	SATMON010	g974781	BLASTN	434	1e-27	84
1314	456	700453369H1	SATMON028	g1814402	BLASTN	436	1e-27	84
1315	456	700621739H1	SATMON034	g1814402	BLASTN	436	1e-27	69
1316	456	700075162H1	SATMON007	g1814402	BLASTN	438	1e-27	85
1317	456	700405040H1	SATMON027	g974781	BLASTN	438	1e-27	72
1318	456	700356893H1	SATMON024	g974781	BLASTN	458	1e-27	82
1319	456	701185493H1	SATMONN06	g886471	BLASTX	72	1e-26	97
1320	456	700206095H1	SATMON003	g974781	BLASTN	421	1e-26	83
1321	456	700083901H1	SATMON011	g1814402	BLASTN	421	1e-26	80
1322	456	LIB3062-026-Q1-K1-D7	LIB3062	g1814402	BLASTN	423	1e-24	81
1323	456	700377277H1	SATMON019	g1814402	BLASTN	357	1e-20	75
1324	456	700201214H1	SATMON003	g2738248	BLASTX	152	1e-18	77
1325	456	700025959H1	SATMON003	g886471	BLASTX	188	1e-18	94
1326	456	700259484H1	SATMON017	g1814402	BLASTN	189	1e-17	82
1327	456	700613969H1	SATMON033	g886470	BLASTN	315	1e-17	90
1328	456	700215602H1	SATMON016	g2738248	BLASTX	158	1e-16	74
1329	456	700438152H1	SATMON026	g2738247	BLASTN	258	1e-16	75
1330	456	700458654H1	SATMON029	g886470	BLASTN	330	1e-16	70
1331	456	700449563H1	SATMON028	g886471	BLASTX	163	1e-15	89
1332	456	700236490H1	SATMON010	g1814402	BLASTN	276	1e-14	89
1333	456	700456927H1	SATMON029	g1814403	BLASTX	119	1e-13	86
1334	456	701180088H1	SATMONN05	g886471	BLASTX	152	1e-13	60
1335	456	700455716H1	SATMON029	g2738248	BLASTX	111	1e-12	67
1336	456	700573520H1	SATMON030	g886471	BLASTX	82	1e-11	84
1337	456	700569938H1	SATMON030	g2738248	BLASTX	132	1e-11	91
1338	456	700551958H1	SATMON022	g2738247	BLASTN	264	1e-11	82
1339	456	700337475H1	SATMON020	g2738247	BLASTN	268	1e-11	83
1340	456	700170827H1	SATMON013	g886471	BLASTX	124	1e-10	77
1341	456	700453382H1	SATMON028	g974781	BLASTN	152	1e-10	85
1342	456	700089211H1	SATMON011	g2738247	BLASTN	245	1e-9	79
1343	456	700103155H1	SATMON010	g2738248	BLASTX	82	1e-8	85
1344	5523	LIB3062-017-Q1-K1-F4	LIB3062	g2738247	BLASTN	932	1e-84	76
1345	5523	700210708H1	SATMON016	g2738247	BLASTN	952	1e-70	79
1346	5523	700219307H1	SATMON011	g1814402	BLASTN	892	1e-65	78
1347	5523	700221188H1	SATMON011	g2738247	BLASTN	867	1e-63	81
1348	5523	700203884H1	SATMON003	g2738247	BLASTN	874	1e-63	77
1349	5523	700218549H1	SATMON011	g2738247	BLASTN	811	1e-58	77
1350	5523	700572845H2	SATMON030	g2738247	BLASTN	785	1e-56	77
1351	5523	700152362H1	SATMON007	g886470	BLASTN	742	1e-52	79
1352	5523	700152138H1	SATMON007	g27382				

2675	-700909658	700909658H1	SOYMON022	g1814402	BLASTN	251	1e-10	91
2676	-700943841	700943841H1	SOYMON024	g886470	BLASTN	549	1e-49	82
2677	-700963223	700963223H1	SOYMON022	g886470	BLASTN	649	1e-45	72
2678	-700974103	700974103H1	SOYMON005	g886470	BLASTN	763	1e-54	80
2679	-700994293	700994293H1	SOYMON011	g974782	BLASTX	97	1e-12	72
2680	-701004816	701004816H1	SOYMON019	g1814402	BLASTN	582	1e-39	84
2681	-701007125	701007125H1	SOYMON019	g1814403	BLASTX	152	1e-13	87
2682	-701008540	701008540H1	SOYMON019	g886470	BLASTN	770	1e-55	74
2683	-701037766	701037766H1	SOYMON029	g1814403	BLASTX	72	1e-10	69
2684	-701062191	701062191H1	SOYMON033	g974781	BLASTN	225	1e-9	79
2685	-701105474	701105474H1	SOYMON036	g974782	BLASTX	164	1e-15	91
2686	-GM14442	LIB3049-056-Q1-E1-B1	LIB3049	g974781	BLASTN	231	1e-10	79
2687	-GM19631	LIB3056-007-Q1-N1-G9	LIB3056	g974781	BLASTN	388	1e-37	78
2688	-GM37189	LIB3051-072-Q1-K1-B5	LIB3051	g1814402	BLASTN	316	1e-15	78
2689	-GM44802	LIB3053-004-Q1-N1-B2	LIB3053	g974782	BLASTX	85	1e-26	90
2690	1382	700683236H1	SOYMON008	g886470	BLASTN	805	1e-58	78
2691	1382	700566434H1	SOYMON002	g886471	BLASTX	162	1e-23	76
2692	15690	701064361H1	SOYMON034	g974781	BLASTN	951	1e-70	84
2693	15690	700847301H1	SOYMON021	g886470	BLASTN	707	1e-66	85
2694	17335	LIB3051-088-Q1-K1-D9	LIB3051	g886470	BLASTN	1285	1e-101	79
2695	17335	701003832H1	SOYMON019	g974781	BLASTN	811	1e-58	77
2696	17335	700864888H1	SOYMON016	g1814402	BLASTN	767	1e-55	79
2697	17335	700672491H1	SOYMON006	g974781	BLASTN	428	1e-46	75
2698	17335	701003457H1	SOYMON019	g1814402	BLASTN	411	1e-29	84
2699	17335	700833672H1	SOYMON019	g974781	BLASTN	445	1e-28	79
2700	17900	700850578H1	SOYMON023	g2738247	BLASTN	905	1e-66	82
2701	17900	701053154H1	SOYMON032	g1814402	BLASTN	725	1e-65	83
2702	17900	700842351H1	SOYMON020	g1814402	BLASTN	878	1e-64	83
2703	17900	700837656H1	SOYMON020	g1814402	BLASTN	841	1e-61	83
2704	17900	700890851H1	SOYMON024	g974781	BLASTN	747	1e-53	80
2705	20688	700908840H1	SOYMON022	g2738247	BLASTN	889	1e-65	82
2706	20688	700908848H1	SOYMON022	g2738247	BLASTN	877	1e-64	81
2707	33542	LIB3051-009-Q1-E1-E5	LIB3051	g974781	BLASTN	1218	1e-92	79
2708	33542	700748773H1	SOYMON013	g974781	BLASTN	669	1e-46	78
2709	33542	700836363H1	SOYMON020	g2738247	BLASTN	596	1e-40	80
2710	4243	701123616H1	SOYMON037	g1814402	BLASTN	995	1e-74	87
2711	4243	700555001H1	SOYMON001	g1814402	BLASTN	975	1e-72	90
2712	4243	701002967H1	SOYMON019	g1814402	BLASTN	950	1e-70	86
2713	4243	700653509H1	SOYMON003	g1814402	BLASTN	539	1e-69	85
2714	4243	701206028H1	SOYMON035	g1814402	BLASTN	938	1e-69	86
2715	4243	700962115H1	SOYMON022	g1814402	BLASTN	923	1e-68	86
2716	4243	700866243H1	SOYMON016	g1814402	BLASTN	909	1e-66	82
2717	4243	700752507H1	SOYMON014	g1814402	BLASTN	887	1e-65	85
2718	4243	701003887H1	SOYMON019	g1814402	BLASTN	863	1e-63	86
2719	4243	700556913H1	SOYMON001	g1814402	BLASTN	865	1e-63	86
2720	4243	701013549H1	SOYMON019	g1814402	BLASTN	867	1e-63	90
2721	4243	701209706H1	SOYMON035	g1814402	BLASTN	871	1e-63	90
2722	4243	701010487H1	SOYMON019	g1814402	BLASTN	529	1e-62	79
2723	4243	700548246H1	SOYMON002	g1814402	BLASTN	553	1e-62	82
2724	4243	701138219H1	SOYMON038	g1814402	BLASTN	594	1e-62	86
2725	4243	700965160H1	SOYMON022	g1814402	BLASTN	852	1e-62	91
2726	4243	701015168H1	SOYMON019	g1814402	BLASTN	855	1e-62	89
2727	4243	701136095H1	SOYMON038	g1814402	BLASTN	839	1e-61	88
2728	4243	700761789H1	SOYMON015	g1814402	BLASTN	845	1e-61	86

2729	4243	701105695H1	SOYMON036	g1814402	BLASTN	835	le-60	87
2730	4243	700991714H1	SOYMON011	g1814402	BLASTN	502	le-59	83
2731	4243	700564223H1	SOYMON002	g1814402	BLASTN	557	le-59	90
2732	4243	700987384H1	SOYMON009	g1814402	BLASTN	803	le-58	86
2733	4243	700833934H1	SOYMON019	g1814402	BLASTN	806	le-58	89
2734	4243	700835181H1	SOYMON019	g1814402	BLASTN	806	le-58	82
2735	4243	700737529H1	SOYMON010	g1814402	BLASTN	810	le-58	92
2736	4243	701012851H1	SOYMON019	g1814402	BLASTN	811	le-58	91
2737	4243	700556592H1	SOYMON001	g1814402	BLASTN	814	le-58	88
2738	4243	700907579H1	SOYMON022	g1814402	BLASTN	781	le-56	89
2739	4243	700961749H1	SOYMON022	g1814402	BLASTN	785	le-56	91
2740	4243	700835239H1	SOYMON019	g1814402	BLASTN	787	le-56	86
2741	4243	700646425H1	SOYMON013	g1814402	BLASTN	772	le-55	89
2742	4243	701123924H1	SOYMON037	g1814402	BLASTN	775	le-55	91
2743	4243	700957759H1	SOYMON022	g1814402	BLASTN	776	le-55	90
2744	4243	700964425H1	SOYMON022	g1814402	BLASTN	777	le-55	86
2745	4243	700962173H1	SOYMON022	g1814402	BLASTN	778	le-55	91
2746	4243	701066293H1	SOYMON034	g1814402	BLASTN	759	le-54	81
2747	4243	700986741H1	SOYMON009	g1814402	BLASTN	589	le-53	84
2748	4243	701212514H1	SOYMON035	g1814402	BLASTN	591	le-53	87
2749	4243	701009195H1	SOYMON019	g1814402	BLASTN	747	le-53	91
2750	4243	701060510H1	SOYMON033	g1814402	BLASTN	748	le-53	84
2751	4243	700848730H1	SOYMON021	g1814402	BLASTN	749	le-53	90
2752	4243	700754870H1	SOYMON014	g1814402	BLASTN	751	le-53	84
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2754	4243	700753283H1	SOYMON014	g1814402	BLASTN	752	le-53	86
2755	4243	700738517H1	SOYMON012	g1814402	BLASTN	636	le-52	89
2756	4243	700833978H1	SOYMON019	g1814402	BLASTN	740	le-52	91
2757	4243	700756424H1	SOYMON014	g1814402	BLASTN	729	le-51	82
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2759	4243	701010103H2	SOYMON019	g1814402	BLASTN	707	le-50	82
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2761	4243	701123062H1	SOYMON037	g1814402	BLASTN	308	le-49	88
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2763	4243	700834566H1	SOYMON019	g1814402	BLASTN	618	le-49	88
2764	4243	700963965H1	SOYMON022	g1814402	BLASTN	685	le-48	78
2765	4243	700986376H1	SOYMON009	g1814402	BLASTN	694	le-48	84
2766	4243	701012708H1	SOYMON019	g1814402	BLASTN	521	le-47	91
2767	4243	700746927H1	SOYMON013	g1814402	BLASTN	547	le-46	78
2768	4243	700997448H1	SOYMON018	g1814402	BLASTN	470	le-45	89
2769	4243	700830667H1	SOYMON019	g1814402	BLASTN	647	le-45	86
2770	4243	700891479H1	SOYMON024	g1814402	BLASTN	654	le-45	88
2771	4243	700562246H1	SOYMON002	g1814402	BLASTN	656	le-45	86
2772	4243	701097325H1	SOYMON028	g18				



2837	550	700952331H1	SOYMON022	g886470	BLASTN	946	1e-69	83
2838	550	700725576H1	SOYMON009	g886470	BLASTN	946	1e-69	85
2839	550	701003602H1	SOYMON019	g2738247	BLASTN	924	1e-68	83
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2843	550	700864264H1	SOYMON016	g1814402	BLASTN	930	1e-68	84
2844	550	700674466H1	SOYMON007	g974781	BLASTN	933	1e-68	83
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2846	550	700654531H1	SOYMON004	g974781	BLASTN	911	1e-67	81
2847	550	700996341H1	SOYMON018	g886470	BLASTN	911	1e-67	83
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2849	550	700751114H1	SOYMON014	g1814402	BLASTN	914	1e-67	84
2850	550	700657606H1	SOYMON004	g1814402	BLASTN	916	1e-67	87
2851	550	700983827H1	SOYMON009	g974781	BLASTN	918	1e-67	85
2852	550	700981249H1	SOYMON009	g1814402	BLASTN	921	1e-67	80
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2855	550	701097045H1	SOYMON028	g886470	BLASTN	601	1e-66	85
2856	550	701012695H1	SOYMON019	g974781	BLASTN	777	1e-66	85
2857	550	700945396H1	SOYMON024	g974781	BLASTN	902	1e-66	85
2858	550	700755390H1	SOYMON014	g974781	BLASTN	903	1e-66	86
2859	550	700967721H1	SOYMON033	g886470	BLASTN	910	1e-66	82
2860	550	700750610H1	SOYMON014	g974781	BLASTN	910	1e-66	84
2861	550	700908231H1	SOYMON022	g974781	BLASTN	527	1e-65	83
2862	550	701003835H1	SOYMON019	g886470	BLASTN	723	1e-65	82
2863	550	700790802H1	SOYMON011	g2738247	BLASTN	891	1e-65	82
2864	550	701053438H1	SOYMON032	g974781	BLASTN	893	1e-65	82
2865	550	701009430H1	SOYMON019	g974781	BLASTN	894	1e-65	85
2866	550	700891415H1	SOYMON024	g1814402	BLASTN	895	1e-65	84
2867	550	701100681H1	SOYMON028	g2738247	BLASTN	482	1e-64	84
2868	550	700893420H1	SOYMON024	g974781	BLASTN	570	1e-64	89
2869	550	700752741H1	SOYMON014	g886470	BLASTN	880	1e-64	82
2870	550	700955938H1	SOYMON022	g1814402	BLASTN	882	1e-64	81
2871	550	701015042H1	SOYMON019	g2738247	BLASTN	886	1e-64	82
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2873	550	700741918H1	SOYMON012	g886470	BLASTN	722	1e-63	84
2874	550	701103319H1	SOYMON028	g974781	BLASTN	792	1e-63	82
2875	550	700833218H1	SOYMON019	g2738247	BLASTN	863	1e-63	81
2876	550	701008071H1	SOYMON019	g886470	BLASTN	867	1e-63	83
2877	550	700832073H1	SOYMON019	g974781	BLASTN	871	1e-63	83
2878	550	700889695H1	SOYMON024	g974781	BLASTN	872	1e-63	83
2879	550	701007489H2	SOYMON019	g974781	BLASTN	873	1e-63	84
2880	550	700895858H1	SOYMON027	g974781	BLASTN	874	1e-63	84
2881	550	700753955H1	SOYMON014	g974781	BLASTN	470	1e-62	87
2882	550	700564433H1	SOYMON002	g886470	BLASTN	757	1e-62	84
2883	550	700963115H1	SOYMON022	g974781	BLASTN	851	1e-62	83
2884	550	700894728H1	SOYMON024	g886470	BLASTN	860	1e-62	84
2885	550	701056915H1	SOYMON033	g886470	BLASTN	862	1e-62	84
2886	550	700741134H1	SOYMON012	g886470	BLASTN	862	1e-62	83
2887	550	700847591H1	SOYMON021	g974781	BLASTN	842	1e-61	84
2888	550	700941253H1	SOYMON024	g2738247	BLASTN	844	1e-61	80
2889	550	701004315H1	SOYMON019	g2738247	BLASTN	846	1e-61	83
2890	550	700895720H1	SOYMON027	g974781	BLASTN	848	1e-61	83

2891	550	701013541H1	SOYMON019	g974781	BLASTN	849	1e-61	84
2892	550	700892552H1	SOYMON024	g886470	BLASTN	719	1e-60	83
2893	550	701141313H1	SOYMON038	g974781	BLASTN	827	1e-60	83
2894	550	701012547H1	SOYMON019	g1814402	BLASTN	831	1e-60	83
2895	550	701008558H1	SOYMON019	g1814402	BLASTN	831	1e-60	83
2896	550	700902022H1	SOYMON027	g2738247	BLASTN	831	1e-60	82
2897	550	700959515H1	SOYMON022	g886470	BLASTN	832	1e-60	81
2898	550	701042630H1	SOYMON029	g1814402	BLASTN	819	1e-59	81
2899	550	700941292H1	SOYMON024	g2738247	BLASTN	820	1e-59	81
2900	550	700788526H1	SOYMON011	g974781	BLASTN	491	1e-58	82
2901	550	700894839H1	SOYMON024	g1814402	BLASTN	498	1e-58	82
2902	550	700865873H1	SOYMON016	g974781	BLASTN	808	1e-58	85
2903	550	701015435H1	SOYMON019	g886470	BLASTN	809	1e-58	80
2904	550	700755960H1	SOYMON014	g2738247	BLASTN	809	1e-58	78
2905	550	700876051H1	SOYMON018	g886470	BLASTN	434	1e-57	81
2906	550	701041327H1	SOYMON029	g886470	BLASTN	499	1e-57	83
2907	550	701098902H1	SOYMON028	g2738247	BLASTN	767	1e-57	77
2908	550	700853392H1	SOYMON023	g886470	BLASTN	793	1e-57	82
2909	550	700872645H1	SOYMON018	g974781	BLASTN	798	1e-57	83
2910	550	700989675H1	SOYMON011	g2738247	BLASTN	800	1e-57	79
2911	550	700753487H1	SOYMON014	g1814402	BLASTN	589	1e-56	83
2912	550	700736276H1	SOYMON010	g1814402	BLASTN	782	1e-56	79
2913	550	700891361H1	SOYMON024	g1814402	BLASTN	783	1e-56	81
2914	550	700829712H1	SOYMON019	g974781	BLASTN	790	1e-56	80
2915	550	LIB3050-019-Q1-K1-A1	LIB3050	g974781	BLASTN	663	1e-55	81
2916	550	701001013H1	SOYMON018	g1814402	BLASTN	768	1e-55	84
2917	550	LIB3028-031-Q1-B1-G12	LIB3028	g886470	BLASTN	775	1e-55	82
2918	550	701212782H1	SOYMON035	g886470	BLASTN	621	1e-54	82
2919	550	701008695H1	SOYMON019	g974781	BLASTN	718	1e-54	80
2920	550	700990972H1	SOYMON011	g1814402	BLASTN	766	1e-54	81
2921	550	700789576H2	SOYMON011	g886470	BLASTN	766	1e-54	80
2922	550	700994266H1	SOYMON011	g1814402	BLASTN	408	1e-53	85
2923	550	700731985H1	SOYMON010	g974781	BLASTN	590	1e-53	81
2924	550	700907927H1	SOYMON022	g886470	BLASTN	612	1e-53	80
2925	550	701012079H1	SOYMON019	g974781	BLASTN	669	1e-53	86
2926	550	700753939H1	SOYMON014	g886470	BLASTN	690	1e-53	78
2927	550	701000754H1	SOYMON018	g974781	BLASTN	745	1e-53	76
2928	550	701040287H1	SOYMON029	g886470	BLASTN	747	1e-53	82
2929	550	700891329H1	SOYMON024	g974781	BLASTN	749	1e-53	79
2930	550	700897258H1	SOYMON027	g886470	BLASTN	752	1e-53	86
2931	550	700944949H1	SOYMON024	g974781	BLASTN	426	1e-52	82
2932	550	701108671H1	SOYMON036	g1814402	BLASTN	731	1e-52	77
2933	550	700905233H1	SOYMON022	g886470	BLASTN	732	1e-52	77
2934	550	700958589H1	SOYMON022	g886470	BLASTN	738	1e-52	80
2935	550	700666436H1	SOYMON005	g2738247	BLASTN	739	1e-52	82
2936	550	700829902H1	SOYMON019	g886470	BLASTN	740	1e-52	82
2937	550	700740110H1	SOYMON012	g974781	BLASTN	742	1e-52	83
2938	550	700989055H1	SOYMON011	g886470	BLASTN	538	1e-50	79
2939	550	701213370H1	SOYMON035	g974781	BLASTN	599	1e-50	83
2940	550	701098072H1	SOYMON028	g974781	BLASTN	709	1e-50	74
2941	550	700896128H1	SOYMON027	g886470	BLASTN	714	1e-50	83
2942	550	701060755H1	SOYMON033	g974781	BLASTN	716	1e-50	74
2943	550	700953594H1	SOYMON022	g1814402	BLASTN	695	1e-49	78
2944	550	701046911H1	SOYMON032	g2738247	BLASTN	687	1e-48	81

2945	550	701065707H1	SOYMON034	g1814402	BLASTN	443	1e-47	84
2946	550	700962114H1	SOYMON022	g886470	BLASTN	678	1e-47	84
2947	550	700831826H1	SOYMON019	g2738247	BLASTN	682	1e-47	77
2948	550	701054296H1	SOYMON032	g886470	BLASTN	454	1e-46	83
2949	550	700888738H1	SOYMON024	g974781	BLASTN	552	1e-46	77
2950	550	700892022H1	SOYMON024	g886470	BLASTN	605	1e-46	81
2951	550	700890275H1	SOYMON024	g2738247	BLASTN	666	1e-46	77
2952	550	LIB3051-006-Q1-K1-G9	LIB3051	g974781	BLASTN	670	1e-45	80
2953	550	700889113H1	SOYMON024	g886470	BLASTN	582	1e-43	81
2954	550	700952720H1	SOYMON022	g886470	BLASTN	611	1e-42	76
2955	550	701014761H1	SOYMON019	g886470	BLASTN	318	1e-41	84
2956	550	700753882H1	SOYMON014	g886470	BLASTN	381	1e-41	79
2957	550	700743792H1	SOYMON012	g1814402	BLASTN	610	1e-41	86
2958	550	700990963H1	SOYMON011	g886470	BLASTN	578	1e-39	77
2959	550	700941880H1	SOYMON024	g2738247	BLASTN	583	1e-39	81
2960	550	700898962H1	SOYMON027	g2738247	BLASTN	571	1e-38	80
2961	550	700990865H1	SOYMON011	g2738247	BLASTN	467	1e-37	77
2962	550	700565779H1	SOYMON002	g886470	BLASTN	557	1e-37	70
2963	550	700993903H1	SOYMON011	g974781	BLASTN	562	1e-37	84
2964	550	700941589H1	SOYMON024	g2738247	BLASTN	550	1e-36	81
2965	550	701052554H1	SOYMON032	g886470	BLASTN	534	1e-35	67
2966	550	700991055H1	SOYMON011	g886470	BLASTN	356	1e-34	79
2967	550	701010438H1	SOYMON019	g2738247	BLASTN	508	1e-33	79
2968	550	700756634H1	SOYMON014	g2738247	BLASTN	494	1e-32	76
2969	550	701042980H1	SOYMON029	g886470	BLASTN	461	1e-29	83
2970	550	700682940H1	SOYMON008	g2738247	BLASTN	466	1e-29	83
2971	550	701049575H1	SOYMON032	g886470	BLASTN	433	1e-27	82
2972	550	700982552H1	SOYMON009	g886470	BLASTN	356	1e-25	78
2973	550	700675637H1	SOYMON007	g886470	BLASTN	375	1e-25	79
2974	550	701142153H1	SOYMON038	g886470	BLASTN	377	1e-22	76
2975	550	700682724H1	SOYMON008	g974781	BLASTN	361	1e-19	88
2976	550	701051764H1	SOYMON032	g1814402	BLASTN	211	1e-17	80
2977	550	700867241H1	SOYMON016	g2738248	BLASTX	152	1e-13	88
2978	550	701054954H1	SOYMON032	g2738248	BLASTX	138	1e-12	86
2979	550	700790450H2	SOYMON011	g974781	BLASTN	238	1e-10	80
2980	550	700653979H1	SOYMON003	g1814403	BLASTX	118	1e-9	92
2981	550	700894218H1	SOYMON024	g2738248	BLASTX	122	1e-9	78
2982	550	700863078H1	SOYMON022	g2738247	BLASTN	236	1e-8	78
2983	5758	701209304H1	SOYMON035	g886470	BLASTN	766	1e-54	84
2984	5758	701106455H1	SOYMON036	g886470	BLASTN	723	1e-51	82
2985	5758	700833538H1	SOYMON019	g1814402	BLASTN	609	1e-43	83
2986	5758	701051425H1	SOYMON032	g886470	BLASTN	629	1e-43	83
2987	5758	700654506H1	SOYMON004	g886470	BLASTN	438	1e-26	70
2988	5758	701047795H1	SOYMON032	g974782	BLASTX	161	1e-15	100
2989	5758	701202409H1	SOYMON035	g1814402	BLASTN	310	1e-15	79
2990	8266	700558628H1	SOYMON001	g886470	BLASTN	780	1e-56	74
2991	8266	701207720H1	SOYMON035	g1814402	BLASTN	766	1e-54	74
2992	8266	700557429H1	SOYMON001	g1814402	BLASTN	728	1e-51	75

# ADENOSYLHOMOCYSTEINASE ( EC 3.3.1.1 )

Seq No.	Cluster ID	CloneID	Library	NCBI gi	Method	Score	P-value	%Ident
1354	-700154280	700154280H1	SATMON007	g170772	BLASTN	229	1e-23	77



1355	-L30594291	LIB3059-032-Q1-K1-A8	LIB3059	g170772	BLASTN	516	1e-74	73
1356	-L30664307	LIB3066-047-Q1-K1-E7	LIB3066	g170772	BLASTN	628	1e-49	70
1357	503	LIB3079-013-Q1-K1-D3	LIB3079	g170772	BLASTN	1505	1e-139	89
1358	503	LIB3062-017-Q1-K1-A10	LIB3062	g170772	BLASTN	1571	1e-129	89
1359	503	LIB3067-001-Q1-K1-D11	LIB3067	g170772	BLASTN	1654	1e-129	89
1360	503	LIB148-060-Q1-E1-B9	LIB148	g170772	BLASTN	1620	1e-126	90
1361	503	LIB3069-043-Q1-K1-G4	LIB3069	g170772	BLASTN	1360	1e-124	84
1362	503	LIB143-006-Q1-E1-F3	LIB143	g170772	BLASTN	831	1e-120	88
1363	503	LIB189-009-Q1-E1-E9	LIB189	g170772	BLASTN	1551	1e-120	90
1364	503	LIB3060-003-Q1-K1-F9	LIB3060	g170772	BLASTN	1538	1e-119	9
1365	503	700089023H1	SATMON011	g170772	BLASTN	1495	1e-115	91
1366	503	LIB143-061-Q1-E1-C9	LIB143	g170772	BLASTN	1474	1e-114	90
1367	503	LIB3069-034-Q1-K1-E5	LIB3069	g170772	BLASTN	1483	1e-114	88
1368	503	LIB143-061-Q1-E1-E5	LIB143	g170772	BLASTN	1440	1e-113	89
1369	503	LIB3067-040-Q1-K1-H5	LIB3067	g170772	BLASTN	1467	1e-113	88
1370	503	LIB3067-048-Q1-K1-A11	LIB3067	g170772	BLASTN	1207	1e-109	86
1371	503	LIB3068-050-Q1-K1-G6	LIB3068	g170772	BLASTN	1279	1e-109	86
1372	503	LIB3069-036-Q1-K1-F6	LIB3069	g170772	BLASTN	1310	1e-109	92
1373	503	LIB3066-047-Q1-K1-H5	LIB3066	g170772	BLASTN	1353	1e-108	86
1374	503	LIB3059-011-Q1-K1-A5	LIB3059	g170772	BLASTN	1401	1e-107	90
1375	503	LIB3067-056-Q1-K1-E11	LIB3067	g170772	BLASTN	957	1e-106	86
1376	503	LIB189-010-Q1-E1-E12	LIB189	g170772	BLASTN	1144	1e-106	88
1377	503	700084426H1	SATMON011	g170772	BLASTN	1368	1e-105	91
1378	503	700086273H1	SATMON011	g170772	BLASTN	1364	1e-104	91
1379	503	700573027H1	SATMON030	g170772	BLASTN	939	1e-103	89
1380	503	700209360H1	SATMON016	g170772	BLASTN	1350	1e-103	89
1381	503	700619916H1	SATMON034	g170772	BLASTN	1050	1e-102	89
1382	503	700086051H1	SATMON011	g170772	BLASTN	1336	1e-102	90
1383	503	LIB3069-034-Q1-K1-C8	LIB3069	g170772	BLASTN	1322	1e-101	86
1384	503	700026324H1	SATMON003	g170772	BLASTN	1328	1e-101	91
1385	503	700104549H1	SATMON010	g170772	BLASTN	836	1e-100	88
1386	503	700622108H1	SATMON034	g170772	BLASTN	1158	1e-100	88
1387	503	700093980H1	SATMON008	g170772	BLASTN	1312	1e-100	90
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1389	503	700095389H1	SATMON008	g170772	BLASTN	1302	1e-99	91
1390	503	LIB3067-055-Q1-K1-D3	LIB3067	g170772	BLASTN	762	1e-98	87
1391	503	LIB3060-054-Q1-K1-F6	LIB3060	g170772	BLASTN	1009	1e-98	83
1392	503	700083339H1	SATMON011	g170772	BLASTN	1282	1e-98	91
1393	503	700102631H1	SATMON010	g170772	BLASTN	1283	1e-98	89
1394	503	700265625H1	SATMON017	g170772	BLASTN	1286	1e-98	90
1395	503	700095002H1	SATMON008	g170772	BLASTN	1289	1e-98	88
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1410	503	700072038H1	SATMON007	g170772	BLASTN	1237	1e-94	89
1411	503	700082967H1	SATMON011	g170772	BLASTN	1239	1e-94	89
1412	503	LIB3068-062-Q1-K1-A3	LIB3068	g170772	BLASTN	1148	1e-93	82
1413	503	700094713H1	SATMON008	g170772	BLASTN	1222	1e-93	88
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1415	503	700242509H1	SATMON010	g170772	BLASTN	1213	1e-92	92
1416	503	700071923H1	SATMON007	g170772	BLASTN	1214	1e-92	90
1417	503	700575314H1	SATMON030	g170772	BLASTN	1149	1e-91	88
1418	503	700086654H1	SATMON011	g170772	BLASTN	1199	1e-91	90
1419	503	700241072H1	SATMON010	g170772	BLASTN	1205	1e-91	91
1420	503	700047361H1	SATMON003	g170772	BLASTN	1110	1e-90	89
1421	503	700217056H1	SATMON016	g170772	BLASTN	1187	1e-90	91
1422	503	LIB3067-005-Q1-K1-F2	LIB3067	g170772	BLASTN	1188	1e-90	84
1423	503	700075495H1	SATMON007	g170772	BLASTN	1193	1e-90	86
1424	503	700084783H1	SATMON011	g170772	BLASTN	1194	1e-90	91
1425	503	LIB143-059-Q1-E1-C3	LIB143	g170772	BLASTN	952	1e-89	84
1426	503	700448818H1	SATMON028	g170772	BLASTN	1174	1e-89	91
1427	503	700159079H1	SATMON012	g170772	BLASTN	1177	1e-89	92
1428	503	700094408H1	SATMON008	g170772	BLASTN	1177	1e-89	92
1429	503	700348440H1	SATMON023	g170772	BLASTN	1182	1e-89	89
1430	503	700077082H1	SATMON007	g170772	BLASTN	1182	1e-89	92
1431	503	700082111H1	SATMON011	g170772	BLASTN	1183	1e-89	89
1432	503	700239752H1	SATMON010	g170772	BLASTN	1164	1e-88	89
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1437	503	700102234H1	SATMON010	g170772	BLASTN	634	1e-85	90
1438	503	700450479H1	SATMON028	g170772	BLASTN	1015	1e-85	90
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1440	503	700218734H1	SATMON011	g170772	BLASTN	1128	1e-85	94
1441	503	700256858H1	SATMON017	g170772	BLASTN	1133	1e-85	90
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1443	503	700105439H1	SATMON010	g170772	BLASTN	1118	1e-84	89
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1446	503	700077492H1	SATMON007	g170772	BLASTN	774	1e-83	88
1447	503	700209295H1	SATMON016	g170772	BLASTN	914	1e-83	89
1448	503	700455826H1	SATMON029	g170772	BLASTN	978	1e-83	91
1449	503	700213370H1	SATMON016	g170772	BLASTN	1110	1e-83	92
1450	503	700352095H1	SATMON023	g170772	BLASTN	1111	1e-83	89
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1452	503	700215872H1	SATMON016	g170772	BLASTN	1113	1e-83	89
1453	503	700073645H1	SATMON007	g170772	BLASTN	1113	1e-83	89
1454	503	700048153H1	SATMON003	g170772	BLASTN	651	1e-82	90
1455	503	700073307H1	SATMON007	g170772	BLASTN	762	1e-82	90
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1459	503	LIB3059-042-Q1-K1-H12	LIB3059	g170772	BLASTN	1090	1e-82	85
1460	503	700238024H1	SATMON010	g170772	BLASTN	1091	1e-82	90
1461	503	700155863H1	SATMON007	g170772	BLASTN	1091	1e-82	93
1462	503	700217890H1	SATMON016	g170772	BLASTN	1093	1e-82	91

1463	503	700239314H1	SATMON010	g170772	BLASTN	1094	1e-82	88
1464	503	700048337H1	SATMON003	g170772	BLASTN	1094	1e-82	92
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1467	503	700209374H1	SATMON016	g170772	BLASTN	1083	1e-81	89
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1469	503	700159326H1	SATMON012	g170772	BLASTN	777	1e-80	91
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1471	503	700210458H1	SATMON016	g170772	BLASTN	856	1e-80	88
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1473	503	700242676H1	SATMON010	g170772	BLASTN	940	1e-80	89
1474	503	700345565H1	SATMON021	g170772	BLASTN	1034	1e-80	89
1475	503	700264733H1	SATMON017	g170772	BLASTN	1044	1e-80	90
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1477	503	700072491H1	SATMON007	g170772	BLASTN	604	1e-79	88
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1483	503	700082291H1	SATMON011	g170772	BLASTN	1043	1e-78	87
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1486	503	700221019H1	SATMON011	g170772	BLASTN	856	1e-77	89
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1491	503	700243831H1	SATMON010	g170772	BLASTN	842	1e-76	89
1492	503	700235671H1	SATMON010	g170772	BLASTN	1019	1e-76	90
1493	503	700087847H1	SATMON011	g170772	BLASTN	1009	1e-75	82
1494	503	700208747H1	SATMON016	g170772	BLASTN	1010	1e-75	89
1495	503	700071795H1	SATMON007	g170772	BLASTN	1011	1e-75	89
1496	503	700157002H1	SATMON012	g170772	BLASTN	1012	1e-75	90
1497	503	700201608H1	SATMON003	g170772	BLASTN	1015	1e-75	85
1498	503	700451541H1	SATMON028	g170772	BLASTN	1015	1e-75	86
1499	503	700212182H1	SATMON016	g170772	BLASTN	1015	1e-75	88
1500	503	700381485H1	SATMON023	g170772	BLASTN	1017	1e-75	91
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1502	503	700093025H1	SATMON008	g170772	BLASTN	996	1e-74	89
1503	503	700017129H1	SATMON001	g170772	BLASTN	998	1e-74	92
1504	503	700216525H1	SATMON016	g170772	BLASTN	1000	1e-74	86
1505	503	700087912H1	SATMON011	g170772	BLASTN	1001	1e-74	91
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1517	503	700094688H1	SATMON008	g170772	BLASTN	975	1e-72	89
1518	503	700170985H1	SATMON013	g170772	BLASTN	979	1e-72	89
1519	503	700451847H1	SATMON028	g170772	BLASTN	872	1e-71	84
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1521	503	700106189H1	SATMON010	g170772	BLASTN	960	1e-71	88
1522	503	700160075H1	SATMON012	g170772	BLASTN	968	1e-71	90
1523	503	700084952H1	SATMON011	g170772	BLASTN	552	1e-70	78
1524	503	700348238H1	SATMON023	g170772	BLASTN	946	1e-70	87
1525	503	700151310H1	SATMON007	g170772	BLASTN	948	1e-70	90
1526	503	700048962H1	SATMON003	g170772	BLASTN	677	1e-69	88
1527	503	700088485H1	SATMON011	g170772	BLASTN	936	1e-69	88
1528	503	700093667H1	SATMON008	g170772	BLASTN	937	1e-69	88
1529	503	700161491H1	SATMON012	g170772	BLASTN	928	1e-68	86
1530	503	700073939H1	SATMON007	g170772	BLASTN	931	1e-68	88
1531	503	700027715H1	SATMON003	g170772	BLASTN	472	1e-67	90
1532	503	700072843H1	SATMON007	g170772	BLASTN	692	1e-67	87
1533	503	700439356H1	SATMON026	g170772	BLASTN	777	1e-67	85
1534	503	700073556H1	SATMON007	g170772	BLASTN	910	1e-67	88
1535	503	700154461H1	SATMON007	g170772	BLASTN	911	1e-67	87
1536	503	700201909H1	SATMON003	g170772	BLASTN	912	1e-67	88
1537	503	700104384H1	SATMON010	g170772	BLASTN	665	1e-66	89
1538	503	700152707H1	SATMON007	g170772	BLASTN	898	1e-66	88
1539	503	700076625H1	SATMON007	g170772	BLASTN	900	1e-66	85
1540	503	700456945H1	SATMON029	g170772	BLASTN	902	1e-66	80
1541	503	700457294H1	SATMON029	g170772	BLASTN	446	1e-65	88
1542	503	700152062H1	SATMON007	g170772	BLASTN	803	1e-65	90
1543	503	700072428H2	SATMON007	g170772	BLASTN	890	1e-65	88
1544	503	700217352H1	SATMON016	g170772	BLASTN	893	1e-65	88
1545	503	700072443H2	SATMON007	g170772	BLASTN	894	1e-65	89
1546	503	700087436H1	SATMON011	g170772	BLASTN	874	1e-64	88
1547	503	700240615H1	SATMON010	g170772	BLASTN	877	1e-64	88
1548	503	700155233H1	SATMON007	g170772	BLASTN	883	1e-64	90
1549	503	700241210H1	SATMON010	g170772	BLASTN	822	1e-63	87
1550	503	700478070H1	SATMON025	g170772	BLASTN	863	1e-63	82
1551	503	700447433H1	SATMON027	g170772	BLASTN	865	1e-63	84
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1553	503	700575250H1	SATMON030	g170772	BLASTN	820	1e-61	84
1554	503	700209283H1	SATMON016	g170772	BLASTN	833	1e-60	86
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1556	503	700241740H1	SATMON010	g170772	BLASTN	814	1e-59	88
1557	503	700026012H1	SATMON003	g170772	BLASTN	815	1e-59	87
1558	503	700151036H1	SATMON007	g170772	BLASTN	816	1e-59	91
1559	503	700207150H1	SATMON017	g170772	BLASTN	818	1e-59	87
1560	503	700217383H1	SATMON016	g170772	BLASTN	824	1e-59	88
1561	503	700618168H1	SATMON033					

1571	503	700424527H1	SATMONN01	g170772	BLASTN	777	1e-55	83
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1575	503	700202577H1	SATMON003	g170772	BLASTN	743	1e-53	86
1576	503	700446645H1	SATMON027	g170772	BLASTN	744	1e-53	87
1577	503	700238061H1	SATMON010	g170772	BLASTN	745	1e-53	86
1578	503	700617325H1	SATMON033	g170772	BLASTN	751	1e-53	91
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1581	503	700083360H1	SATMON011	g170772	BLASTN	739	1e-52	87
1582	503	700236915H1	SATMON010	g170772	BLASTN	740	1e-52	87
1583	503	700074780H1	SATMON007	g170772	BLASTN	528	1e-51	83
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1586	503	700165354H1	SATMON013	g170772	BLASTN	728	1e-51	86
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1588	503	700153619H1	SATMON007	g170772	BLASTN	694	1e-49	86
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1590	503	700155530H1	SATMON007	g170772	BLASTN	686	1e-48	86
1591	503	700221207H1	SATMON011	g170772	BLASTN	692	1e-48	86
1592	503	700264257H1	SATMON017	g170772	BLASTN	672	1e-47	87
1593	503	700152216H1	SATMON007	g170772	BLASTN	659	1e-46	88
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1595	503	700156027H1	SATMON007	g170772	BLASTN	655	1e-45	93
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1597	503	700623795H1	SATMON034	g170772	BLASTN	401	1e-44	88
1598	503	700150581H1	SATMON007	g170772	BLASTN	640	1e-44	88
1599	503	700151970H1	SATMON007	g170772	BLASTN	645	1e-44	87
1600	503	700151780H1	SATMON007	g170772	BLASTN	628	1e-43	88
1601	503	700575547H1	SATMON030	g170772	BLASTN	614	1e-42	90
1602	503	LIB143-026-Q1-E1-A5	LIB143	g170772	BLASTN	633	1e-42	77
1603	503	700347945H1	SATMON023	g170772	BLASTN	586	1e-40	84
1604	503	700074416H1	SATMON007	g170772	BLASTN	589	1e-40	86
1605	503	700352990H1	SATMON024	g170772	BLASTN	575	1e-39	92
1606	503	700156025H1	SATMON007	g170772	BLASTN	568	1e-38	91
1607	503	700432072H1	SATMONN01	g170772	BLASTN	374	1e-37	84
1608	503	700354249H1	SATMON024	g170772	BLASTN	528	1e-35	93
1609	503	700617977H1	SATMON033	g170772	BLASTN	535	1e-35	87
1610	503	700218312H1	SATMON016	g170772	BLASTN	236	1e-33	91
1611	503	700456080H1	SATMON029	g170772	BLASTN	513	1e-33	83
1612	503	700349395H1	SATMON023	g170772	BLASTN	500	1e-32	91
1613	503	700051637H1	SATMON003	g170772	BLASTN	249	1e-31	86
1614	503	700202153H1	SATMON003	g170772	BLASTN	474	1e-30	91
1615	503	700256951H1	SATMON017	g170772	BLASTN	454	1e-29	86
1616	503	700150542H1	SATMON007	g170772	BLASTN	343	1e-28	76
1617	503	700151629H1	SATMON007	g170772	BLASTN	445	1e-28	85
1618	503	700446654H1	SATMON027	g170772	BLASTN	437	1e-27	84
1619	503	700155814H1	SATMON007	g170772	BLASTN	428	1e-26	88
1620	503	700161019H1	SATMON012	g2588780	BLASTN	417	1e-25	92
1621	503	700377236H1	SATMON019	g170772	BLASTN	402	1e-24	84
1622	503	LIB3067-036-Q1-K1-A4	LIB3067	g1220121	BLASTN	224	1e-22	84
1623	503	700158933H1	SATMON012	g170772	BLASTN	368	1e-21	90
1624	503	700155365H1	SATMON007	g170772	BLASTN	347	1e-20	91



3041	16	701065379H1	SOYMON034	g535583	BLASTN	1022	1e-76	86
3042	16	701209645H1	SOYMON035	g170772	BLASTN	795	1e-75	86
3043	16	701055017H1	SOYMON032	g1220121	BLASTN	877	1e-75	86
3044	16	701015213H1	SOYMON019	g535583	BLASTN	1008	1e-75	87
3045	16	700982770H1	SOYMON009	g1220121	BLASTN	1009	1e-75	85
3046	16	701212420H1	SOYMON035	g535583	BLASTN	1012	1e-75	86
3047	16	700977916H1	SOYMON009	g170772	BLASTN	700	1e-74	89
3048	16	700974401H1	SOYMON005	g1220121	BLASTN	857	1e-74	89
3049	16	700645749H1	SOYMON010	g170772	BLASTN	996	1e-74	82
3050	16	700646620H1	SOYMON014	g170772	BLASTN	996	1e-74	89
3051	16	701126103H1	SOYMON037	g170772	BLASTN	1000	1e-74	88
3052	16	700978001H1	SOYMON009	g170772	BLASTN	563	1e-73	89
3053	16	700561987H1	SOYMON002	g1220121	BLASTN	782	1e-73	87
3054	16	701101526H1	SOYMON028	g1220121	BLASTN	810	1e-73	88
3055	16	700562680H1	SOYMON002	g170772	BLASTN	985	1e-73	87
3056	16	700560521H1	SOYMON001	g170772	BLASTN	988	1e-73	80
3057	16	701055544H1	SOYMON032	g170772	BLASTN	992	1e-73	88
3058	16	701061418H1	SOYMON033	g170772	BLASTN	993	1e-73	87
3059	16	701049514H1	SOYMON032	g1220121	BLASTN	884	1e-72	88
3060	16	700646215H1	SOYMON012	g170772	BLASTN	971	1e-72	89
3061	16	701014875H1	SOYMON019	g535583	BLASTN	973	1e-72	87
3062	16	700874718H1	SOYMON018	g169662	BLASTN	974	1e-72	86
3063	16	700897796H1	SOYMON027	g1220121	BLASTN	977	1e-72	87
3064	16	700548019H1	SOYMON001	g170772	BLASTN	547	1e-71	87
3065	16	700904875H1	SOYMON022	g535583	BLASTN	963	1e-71	88
3066	16	701106315H1	SOYMON036	g170772	BLASTN	557	1e-70	90
3067	16	700745023H1	SOYMON013	g407411	BLASTN	949	1e-70	85
3068	16	701120348H1	SOYMON037	g170772	BLASTN	956	1e-70	86
3069	16	701124508H1	SOYMON037	g170772	BLASTN	578	1e-69	84
3070	16	700956183H1	SOYMON022	g1220121	BLASTN	625	1e-69	87
3071	16	700894619H1	SOYMON024	g535583	BLASTN	828	1e-69	85
3072	16	700892392H1	SOYMON024	g535583	BLASTN	936	1e-69	85
3073	16	700730676H1	SOYMON009	g535583	BLASTN	939	1e-69	86
3074	16	700728913H1	SOYMON009	g407411	BLASTN	940	1e-69	87
3075	16	700845737H1	SOYMON021	g1220121	BLASTN	528	1e-68	88
3076	16	700990961H1	SOYMON011	g170772	BLASTN	582	1e-68	85
3077	16	700983443H1	SOYMON009	g170772	BLASTN	922	1e-68	86
3078	16	701120754H1	SOYMON037	g170772	BLASTN	923	1e-68	88
3079	16	700900486H1	SOYMON027	g1220121	BLASTN	924	1e-68	83
3080	16	701133574H2	SOYMON038	g170772	BLASTN	929	1e-68	88
3081	16	700900924H1	SOYMON027	g170772	BLASTN	931	1e-68	88
3082	16	701056706H1	SOYMON032	g170772	BLASTN	486	1e-67	89
3083	16	701110051H1	SOYMON036	g170772	BLASTN	910	1e-67	88
3084	16	701136325H1	SOYMON038	g170772	BLASTN	915	1e-67	87
3085	16	700750809H1	SOYMON014	g170772	BLASTN	900	1e-66	88
3086	16	700686607H1	SOYMON008	g170772	BLASTN	901	1e-66	88
3087	16	700848261H1	SOYMON021	g535583	BLASTN	905	1e-66	87
3088	16	700686634H1	SOYMON008	g170772	BLASTN	905	1e-66	88
3089	16	700891285H1	SOYMON024	g170772	BLASTN	906	1e-66	88
3090	16	700560291H1	SOYMON001	g170772	BLASTN	906	1e-66	88
3091	16	700752975H1	SOYMON014	g170772	BLASTN	907	1e-66	89
3092	16	701006013H2	SOYMON019	g170772	BLASTN	908	1e-66	87
3093	16	700974038H1	SOYMON005	g1220121	BLASTN	631	1e-65	88
3094	16	701047024H1	SOYMON032	g170772	BLASTN	707	1e-65	89

3095	16	700900409H1	SOYMON027	g170772	BLASTN	737	1e-65	83
3096	16	701137320H1	SOYMON038	g170772	BLASTN	769	1e-65	88
3097	16	700978805H1	SOYMON009	g170772	BLASTN	886	1e-65	84
3098	16	700726195H1	SOYMON009	g1220121	BLASTN	889	1e-65	87
3099	16	700661112H1	SOYMON005	g170772	BLASTN	661	1e-64	85
3100	16	700989712H1	SOYMON011	g170772	BLASTN	788	1e-64	88
3101	16	700752287H1	SOYMON014	g170772	BLASTN	875	1e-64	85
3102	16	700964226H1	SOYMON022	g170772	BLASTN	877	1e-64	88
3103	16	700847346H1	SOYMON021	g170772	BLASTN	880	1e-64	83
3104	16	700756428H1	SOYMON014	g170772	BLASTN	883	1e-64	88
3105	16	701049928H1	SOYMON032	g170772	BLASTN	885	1e-64	82
3106	16	700848652H1	SOYMON021	g170772	BLASTN	477	1e-63	89
3107	16	700898929H1	SOYMON027	g1220121	BLASTN	514	1e-63	87
3108	16	700903523H1	SOYMON022	g170772	BLASTN	527	1e-63	82
3109	16	700983745H1	SOYMON009	g1220121	BLASTN	863	1e-63	88
3110	16	700890587H1	SOYMON024	g170772	BLASTN	865	1e-63	86
3111	16	700969917H1	SOYMON005	g407411	BLASTN	868	1e-63	83
3112	16	700808487H1	SOYMON024	g170772	BLASTN	870	1e-63	88
3113	16	700749968H1	SOYMON013	g170772	BLASTN	871	1e-63	87
3114	16	700751254H1	SOYMON014	g170772	BLASTN	575	1e-62	87
3115	16	701014277H1	SOYMON019	g170772	BLASTN	739	1e-62	86
3116	16	700853635H1	SOYMON023	g170772	BLASTN	854	1e-62	87
3117	16	700752357H1	SOYMON014	g170772	BLASTN	856	1e-62	88
3118	16	700754523H1	SOYMON014	g170772	BLASTN	856	1e-62	92
3119	16	700982153H1	SOYMON009	g170772	BLASTN	400	1e-61	82
3120	16	700958283H1	SOYMON022	g170772	BLASTN	839	1e-61	87
3121	16	700980911H1	SOYMON009	g170772	BLASTN	842	1e-61	82
3122	16	700788112H1	SOYMON011	g170772	BLASTN	844	1e-61	83
3123	16	701005927H1	SOYMON019	g170772	BLASTN	849	1e-61	88
3124	16	700756443H1	SOYMON014	g170772	BLASTN	849	1e-61	88
3125	16	700658914H1	SOYMON004	g1220121	BLASTN	468	1e-60	85
3126	16	701135266H1	SOYMON038	g170772	BLASTN	827	1e-60	87
3127	16	700982179H1	SOYMON009	g170772	BLASTN	827	1e-60	82
3128	16	700754593H1	SOYMON014	g170772	BLASTN	832	1e-60	81
3129	16	700831723H1	SOYMON019	g170772	BLASTN	814	1e-59	91
3130	16	700986775H1	SOYMON009	g170772	BLASTN	815	1e-59	92
3131	16	700755219H1	SOYMON014	g170772	BLASTN	820	1e-59	84
3132	16	701015494H1	SOYMON019	g170772	BLASTN	822	1e-59	88
3133	16	701008473H1	SOYMON019	g170772	BLASTN	823	1e-59	87
3134	16	700754981H1	SOYMON014	g170772	BLASTN	824	1e-59	88
3135	16	700870790H1	SOYMON018	g170772	BLASTN	825	1e-59	81
3136	16	700833069H1	SOYMON019	g170772	BLASTN	806	1e-58	86
3137	16	700680127H2	SOYMON008	g535583	BLASTN	807	1e-58	86
3138	16	701015374H1	SOYMON019	g170772	BLASTN	807	1e-58	87
3139	16	700872895H1	SOYMON018	g535583	BLASTN	808	1e-58	88
3140	16	701137912H1	SOYMON038	g170772	BLASTN	374	1e-57	83
3141	16	700984063H1	SOYMON009	g1220121	BLASTN	575	1e-57	79
3142	16	700991988H1	SOYMON011	g170772	BLASTN	791	1e-57	82
3143	16	700873915H1	SOYMON018	g170772	BLASTN	793	1e-57	88
3144	16	700978721H1	SOYMON009	g170772	BLASTN	797	1e-57	78
3145	16	701213679H1	SOYMON035	g170772	BLASTN	798	1e-57	88
3146	16	701102954H1	SOYMON028	g170772	BLASTN	799	1e-57	79
3147	16	700888289H1	SOYMON024	g170772	BLASTN	712	1e-56	91
3148	16	700962086H1	SOYMON022	g170772	BLASTN	783	1e-56	88



3149	16	701052227H1	SOYMON032	g535583	BLASTN	788	1e-56	86
3150	16	700755177H1	SOYMON014	g170772	BLASTN	768	1e-55	88
3151	16	701123136H1	SOYMON037	g170772	BLASTN	771	1e-55	82
3152	16	700979067H1	SOYMON009	g170772	BLASTN	776	1e-55	90
3153	16	700755513H1	SOYMON014	g170772	BLASTN	759	1e-54	92
3154	16	700756639H1	SOYMON014	g170772	BLASTN	761	1e-54	81
3155	16	700554077H1	SOYMON001	g170772	BLASTN	371	1e-53	86
3156	16	700653194H1	SOYMON003	g170772	BLASTN	395	1e-53	86
3157	16	701110348H1	SOYMON036	g170772	BLASTN	743	1e-53	81
3158	16	700753973H1	SOYMON014	g170772	BLASTN	743	1e-53	87
3159	16	701011009H1	SOYMON019	g535583	BLASTN	733	1e-52	81
3160	16	700739086H1	SOYMON012	g170772	BLASTN	456	1e-51	87
3161	16	700740140H1	SOYMON012	g170772	BLASTN	723	1e-51	89
3162	16	700565040H1	SOYMON002	g170772	BLASTN	729	1e-51	74
3163	16	701148186H1	SOYMON031	g535583	BLASTN	665	1e-50	85
3164	16	701142734H1	SOYMON038	g535583	BLASTN	670	1e-47	86
3165	16	700754441H1	SOYMON014	g170772	BLASTN	385	1e-46	93
3166	16	701102588H1	SOYMON028	g1220121	BLASTN	662	1e-46	89
3167	16	700900656H1	SOYMON027	g535583	BLASTN	635	1e-44	87
3168	16	700974207H1	SOYMON005	g535583	BLASTN	641	1e-44	85
3169	16	700982081H1	SOYMON009	g170772	BLASTN	494	1e-43	78
3170	16	701130026H1	SOYMON037	g1220121	BLASTN	482	1e-42	86
3171	16	701009720H1	SOYMON019	g170772	BLASTN	621	1e-42	87
3172	16	700962602H1	SOYMON022	g170772	BLASTN	357	1e-40	91
3173	16	700724934H1	SOYMON009	g535583	BLASTN	561	1e-40	81
3174	16	700729305H1	SOYMON009	g535583	BLASTN	544	1e-39	82
3175	16	701210054H1	SOYMON035	g535583	BLASTN	569	1e-38	85
3176	16	700790192H1	SOYMON011	g535583	BLASTN	293	1e-37	83
3177	16	700984076H1	SOYMON009	g170772	BLASTN	198	1e-35	88
3178	16	700726562H1	SOYMON009	g170772	BLASTN	307	1e-34	78
3179	16	701211376H1	SOYMON035	g170772	BLASTN	524	1e-34	86
3180	16	700753085H1	SOYMON014	g2588780	BLASTN	358	1e-33	76
3181	16	700727993H1	SOYMON009	g535583	BLASTN	465	1e-32	84
3182	16	700561072H1	SOYMON001	g170772	BLASTN	473	1e-30	83
3183	16	701211464H1	SOYMON035	g170772	BLASTN	464	1e-28	81
3184	16	701098045H1	SOYMON028	g169660	BLASTN	386	1e-21	78
3185	16	700945233H1	SOYMON024	g407412	BLASTX	150	1e-18	87
3186	16	700752655H1	SOYMON014	g758247	BLASTX	172	1e-16	94
3187	16	700735356H1	SOYMON010	g758247	BLASTX	152	1e-14	100
3188	16	700683995H1	SOYMON008	g758247	BLASTX	106	1e-13	90
3189	16	700658760H1	SOYMON004	g1857024	BLASTX	123	1e-13	63
3190	16	700762885H1	SOYMON015	g1857024	BLASTX	134	1e-13	89
3191	16	700755740H1	SOYMON014	g170773	BLASTX	149	1e-13	100
3192	16	700854969H1	SOYMON023	g170772	BLASTN	178	1e-12	80
3193	16	701143036H1	SOYMON038	g169661	BLASTX	113	1e-8	83
3194	18409	700786561H1	SOYMON011	g535583	BLASTN	992	1e-73	85
3195	18409	701008057H1	SOYMON019	g535583	BLASTN	831	1e-60	87
3196	18409	701037442H1	SOYMON029	g535583	BLASTN	669	1e-46	86
3197	18409	700942865H1	SOYMON024	g535583	BLASTN	464	1e-32	86
3198	7322	700651524H1	SOYMON003	g170772	BLASTN	466	1e-65	80
3199	7322	700565758H1	SOYMON002	g170772	BLASTN	450	1e-63	81

**THE** **NEW** **YORK** **PUBLIC** **LIBRARY**

CYSTATHIONINE $\gamma$ -LYASE ( EC 4.4.1.1 )								
Seq No.	Cluster ID	CloneID	Library	NCBI gi	Method	Score	P-value	%Ident
1633	-700260027	700260027H1	SATMON017	g169475	BLASTX	112	1e-10	75
1634	1228	700027629H1	SATMON003	g169475	BLASTX	189	1e-19	87
3203	-700750583	700750583H1	SOYMON014	g169475	BLASTX	149	1e-13	78
3204	12502	LIB3051-069-Q1-K1-E6	LIB3051	g2641242	BLASTX	86	1e-30	38

Seq No.	Cluster ID	CloneID	Library	NCBI gi	Method	Score	P-value	%Ident
3200	12502	701135185H1	SOYMON038	g1628606	BLASTX	100	1e-10	48
3201	12502	701042913H1	SOYMON029	g2605905	BLASTX	110	1e-9	42
3202	12502	701059330H1	SOYMON033	g2605905	BLASTX	110	1e-9	42

**\*Table Headings**  
**Cluster ID**

A cluster ID is arbitrarily assigned to all of those clones which belong to the same cluster at a given stringency and a particular clone will belong to only one cluster at a given stringency. If a cluster contains only a single clone (a “singleton”), then the cluster ID number will be negative, with an absolute value equal to the clone ID number of its single member. The cluster ID entries in the table refer to the cluster with which the particular clone in each row is associated.

**Clone ID**

The clone ID number refers to the particular clone in the PhytoSeq database. Each clone ID entry in the table refers to the clone whose sequence is used for (1) the sequence comparison whose scores are presented and/or (2) assignment to the particular cluster which is presented. Note that a clone may be included in this table even if its sequence comparison scores fail to meet the minimum standards for similarity. In such a case, the clone is included due solely to its association with a particular cluster for which sequences of one or more other member clones possess the required level of similarity.

**Library**

The library ID refers to the particular cDNA library from which a given clone is obtained. Each cDNA library is associated with the particular tissue(s), line(s) and developmental stage(s) from which it is isolated.

**NCBI gi**

Each sequence in the GenBank public database is arbitrarily assigned a unique NCBI gi (National Center for Biotechnology Information GenBank Identifier) number. In this table, the

NCBI gi number which is associated (in the same row) with a given clone refers to the particular GenBank sequence which is used in the sequence comparison. This entry is omitted when a clone is included solely due to its association with a particular cluster.

### **Method**

The entry in the "Method" column of the table refers to the type of BLAST search that is used for the sequence comparison. "CLUSTER" is entered when the sequence comparison scores for a given clone fail to meet the minimum values required for significant similarity. In such cases, the clone is listed in the table solely as a result of its association with a given cluster for which sequences of one or more other member clones possess the required level of similarity.

### **Score**

Each entry in the "Score" column of the table refers to the BLAST score that is generated by sequence comparison of the designated clone with the designated GenBank sequence using the designated BLAST method. This entry is omitted when a clone is included solely due to its association with a particular cluster. If the program used to determine the hit is HMMSW then the score refers to HMMSW score.

### **P-Value**

The entries in the P-Value column refer to the probability that such matches occur by chance.

### **%Ident**

The entries in the "%Ident" column of the table refer to the percentage of identically matched nucleotides (or residues) that exist along the length of that portion of the sequences which is aligned by the BLAST comparison to generate the statistical scores presented. This entry is omitted when a clone is included solely due to its association with a particular cluster.